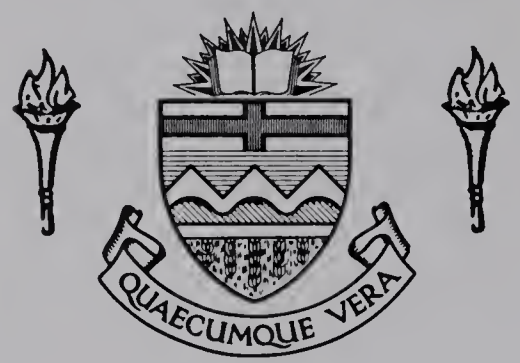


The University of Alberta
Printing Department
Edmonton, Alberta

Grad St

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR ..... Donald Golko .....

TITLE OF THESIS ..... The Transport of Ephedrine by Rabbit Atria .....

.....

.....

DEGREE FOR WHICH THESIS WAS PRESENTED ..... Master of Science .....

YEAR THIS DEGREE GRANTED ..... 1975 .....

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

THE UNIVERSITY OF ALBERTA

THE TRANSPORT OF EPHEDRINE BY RABBIT ATRIA

by

© DONALD GOLKO

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA, CANADA

FALL, 1975

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled *THE TRANSPORT OF EPHEDRINE BY RABBIT ATRIA* submitted by Donald Golko in partial fulfilment of the requirements for the degree of Master of Science.

TO MY WIFE GLORIA AND

MY CHILDREN LISA AND GREGORY

ABSTRACT

Pieces of rabbit atria when incubated in Krebs solution containing 5 x 10^{-7} M ^{14}C-ephedrine accumulated the amine to a level 2.7 times greater than that in the incubation medium. The tissue to medium concentration ratio decreased only slightly with 10-fold or greater increases in the concentration of the amine in the medium. Ephedrine was accumulated by the tissue at a uniform rate even at high concentrations without any indication of maximum velocity being approached. The uptake of ephedrine could not therefore be described by the Michealis-Menten equation.

The transport of ephedrine was partially inhibited by a wide variety of amines. However, high concentrations of amines were required to produce significant inhibition. The amines lacking phenolic hydroxyl groups, *e.g.*, amphetamine and phentermine, and therefore considered to be more lipid soluble, exerted the greatest inhibitory effect on ephedrine accumulation. Unlike the transport system for noradrenaline, the accumulation of ephedrine was not stereochemically specific.

Cocaine and desipramine slightly reduced ephedrine accumulation. Estradiol and testosterone, both potent Uptake$_2$ inhibitors, diminished ephedrine accumulation but not totally. Corticosterone, another potent Uptake$_2$ inhibitor, was without effect. The β-haloalkylamines, phenoxybenzamine and SKF-550, also potent Uptake$_2$ inhibitors were also ineffective. Oxytetracycline, reported to inhibit the binding of catecholamines to collagen did not inhibit the accumulation of ephedrine.

Oubain reduced the accumulation slightly but only when high

concentrations were used. Sodium enhanced the accumulation of ephedrine but was not an absolute requirement and secondly a large fraction of the amine accumulated in the presence of low sodium. No other ion was able to substitute for sodium. Removing potassium from the incubation medium did not decrease the accumulation of ephedrine. Accumulation of ephedrine was unaltered by metabolic inhibitors either by themselves or in a combination to inhibit both aerobic and anaerobic glycolysis. These results indicated that active transport was not involved.

The most important observation of this study was that chemical denervation by 6-hydroxydopamine failed to affect the accumulation of ephedrine. This indicated that the transport of ephedrine was not dependent upon the intactness of the sympathetic nervous system.

In this study several features of ephedrine transport emerged. Transport of ephedrine by $Uptake_1$ is unlikely since chemical denervation by 6-hydroxydopamine failed to inhibit ephedrine accumulation. Active transport with and without co-transport with Na^+ is unlikely since ephedrine accumulation was non-saturable, did not require metabolic energy and was not affected by potassium-free medium. Accumulation of ephedrine by $Uptake_2$ is also unlikely since normetanephrine, phenoxybenzamine and SKF-550 failed to inhibit ephedrine uptake. Oxytetracycline did not inhibit ephedrine accumulation and thus binding of ephedrine to collagen is unlikely. The possibility that ephedrine diffuses passively into the tissue and binds to non-specific sites must be considered. This view is strongly supported by the differences found between its transport and that of noradrenaline. The accumulation of ephedrine was non-saturable, did not exhibit stereochemical specificity, T/M ratios were only slightly diminished with 10-fold or greater increases in amine concentration,

unaffected by most Uptake$_2$ inhibitors and not impaired by a combined inhibition of aerobic and anaerobic glycolysis. The high concentration of amine, *e.g.*, phentermine and metaraminol; and other drugs, *e.g.*, oubain and cocaine required to achieve an inhibitory effect on ephedrine transport is further supporting evidence for a passive diffusion process.

ACKNOWLEDGEMENTS

I thank my supervisor, Dr. David M. Paton, for his help and guidance during the progress of this work. His criticisms and suggestions are also acknowledged.

I also thank Mr. F.E. Loeffler and Mr. K. Burt for the preparation of the figures and Gladys Zuber for her typing abilities.

For financial support during this study, I thank the University of Alberta for a Teaching Assistantship.

TABLE OF CONTENTS

		PAGE
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	8
	(A) General	8
	(B) Kinetics	13
	(C) Stereochemical Specificity of Transport	14
	(D) Temperature Dependence	15
	(E) Effect of Metabolic Inhibition	15
	(F) Na^+ and K^+ Requirements for Transport	17
	(G) Effect of Oubain	18
	(H) Objective of Thesis	21
III.	METHODS AND MATERIALS	22
	(A) Preparation of Tissue	22
	(B) Determination of ^{14}C-Content in Tissue and Media	22
	(C) Purity of ^{14}C-Ephedrine	23
	(D) Chromatographic Analysis of ^{14}C-Ephedrine	24
	(E) Solutions	27
	(F) Drugs and Chemicals	27
	(G) Preparation of Denervated Tissue	29
	(H) Expression of Results	29
	(I) Metabolism of ^{14}C-Ephedrine in the tissue	29
	(J) Statistical Analysis	30

PAGE

IV. RESULTS---------- 32

(A) *Effect of Drugs and Conditions on Tissue Weight Loss*-- 32

(B) *Ephedrine Uptake*---------- 32

(C) *Effect of Concentration on Ephedrine Uptake*---------- 34

(D) *Initial Rates of Ephedrine Accumulation*---------- 38

(E) *Effect of Various Amines on Ephedrine Accumulation*---- 38

(F) *Inhibition of Ephedrine Uptake by Cocaine and Desipramine*---------- 44

(G) *The Effect of Other Drugs on Ephedrine Accumulation*--- 47

(H) *Effect of Exposure to Oubain*---------- 47

(I) *Effect of Na^+ and K^+ on Ephedrine Accumulation*-------- 49

(J) *Effect of Temperature on Ephedrine Accumulation*------- 53

(K) *Effect of Metabolic Inhibitors on Ephedrine Uptake*---- 56

(L) *Effect of 6-Hydroxydopamine Pretreatment on Ephedrine Accumulation*---------- 56

V. DISCUSSION---------- 61

VI. BIBLIOGRAPHY---------- 71

LIST OF TABLES

TABLE		PAGE
1	Effect of drugs and conditions on tissue weight loss----	33
2	Effect of various amines on ephedrine accumulation------	41
3	Effects of phenolic hydroxyl groups on ephedrine uptake----------	43
4	Effect of the isomers of ephedrine and amphetamine on ephedrine uptake----------	45
5	Effect of other drugs on ephedrine accumulation---------	48
6	Inability of ions to substitute for Na^+ in the transport of ephedrine by rabbit atria----------	52
7	Effect of K^+-free media and low temperature----------	54
8	Effect of metabolic inhibitors on ephedrine accumulation----------	57
9	Effect of denervation on ephedrine accumulation---------	59
10	Effect of denervation on metaraminol accumulation-------	60

LIST OF FIGURES

FIGURE		PAGE
1	Chromatographic analysis of ^{14}C-ephedrine working solution	25
2	Chromatographic analysis of carrier ephedrine and phenylpropanolamine	26
3	Chromatographic analysis of pooled tissue extracts	31
4	^{14}C-Ephedrine accumulation by rabbit atrial pieces	35
5	Effect of concentration on ephedrine uptake by rabbit atria	36
6	T/M ratio at different ephedrine concentrations	37
7	Initial rates of ephedrine accumulation	39
8	Effect of phentermine on ephedrine accumulation	42
9	Effect of cocaine and desipramine on ephedrine accumulation	46
10	Effect of oubain on ephedrine accumulation	50
11	Effect of varying sodium concentration on the transport of ^{14}C-ephedrine	51
12	Effect of temperature on ephedrine accumulation	55

I. INTRODUCTION

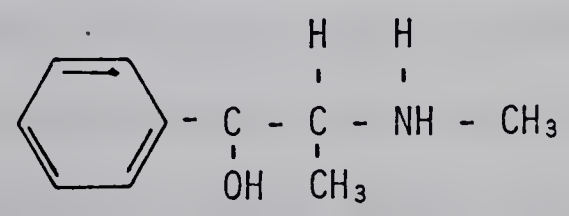
Ephedrine is the crystalline alkaloid which is the active principle of the ancient Chinese herb, Ma Huang, obtained from the Ephedra plant. For some 5000 years, Ma Huang has been used by the Chinese as a diaphoretic, circulatory stimulant, antipyretic, cough sedative, and as an ingredient of many prescriptions.

The Japanese were the first to pioneer the development of a useful drug from this ancient herb. Yamanoshi in 1885 was the first to isolate and obtain a crystalline but impure substance. After his death, Nagai (1887) obtained the alkaloid in pure form, named it ephedrine and was the first to make scientific investigations with it. Physiological investigations with Nagai's ephedrine were first carried out by Miura (1887). This study demonstrated the mydriatic action of the drug, and as a result ephedrine was introduced to medicine as a new mydriatic but was regarded to be very toxic. For the next three decades the drug was used only for ophthalmologic purposes. Several Japanese investigators, Amatsu and Kubota (1918), and Hirose (1915), demonstrated the adrenaline-like effects of ephedrine. As a result of their work the Japanese marketed an ephedrine-containing preparation to be used in the treatment of asthma, a condition that can be relieved by adrenaline. No publications, however, were made of the results obtained.

The work of Chen and Schmidt (1925) was largely responsible for the elevation of ephedrine from obscurity to widespread use in Western medicine. They confirmed that the effects of ephedrine were physiologically very similar to adrenaline but more prolonged and less toxic; the

intestine was relaxed, the uterus stimulated, and mydriasis occurred after local or systemic application. The drug differed from adrenaline in that it was found to be stable in solution and effectively absorbed when applied orally, subcutaneously, or intramuscularly. A monograph prepared by Chen and Schmidt (1930) reviews in some detail the early investigations and findings with ephedrine.

The structural formula of ephedrine is shown below and the similarity to adrenaline and noradrenaline is evident. The graphic formula of the drug contains two assymetric carbon atoms making possible two sets of stereo-isomers. These are designated as the erythro isomers, (-)-, and (+)-ephedrine; and the threo-isomers, (-)-, and (+)-pseudo-ephedrine. Only two, (-)-ephedrine and (+)-pseudoephedrine occur in nature. The isomer, (-)-ephedrine is a waxy solid having a melting point of 34°C. It is soluble in water, alcohol, ether and chloroform. Solutions are strongly alkaline. The pKa of ephedrine is 9.58 (Leffler *et al.*, 1951). Hydrochloride derivatives of (-)-, or (±)-ephedrine are most commonly used for laboratory and clinical purposes. Both hydrochlorides are white crystalline compounds which are soluble in water and alcohol but practically insoluble in ether. The melting point of (-)-ephedrine hydrochloride is 216-220°C and that of (±)-ephedrine, 187-188°C.



Metabolism of ephedrine varies from species to species. The amine is metabolically relatively stable in man who excretes the drug mostly unchanged (Wilkinson and Beckett, 1968). Axelrod (1953) studied

the excretion of ephedrine metabolites in rats, dogs, guinea pigs and rabbits. The rat excreted the amine largely unchanged and a small portion para-hydroxylated. Ephedrine is rapidly N-demethylated to the metabolically stable phenylpropanolamine (norephedrine) in the dog and is excreted as such. Large amounts of the demethylated metabolite, phenylpropanolamine, and small amounts of the para-hydroxylated compounds are excreted by the guinea pig. Ephedrine is extensively metabolized by rabbits which have the necessary enzymes to N-demethylate and deaminate ephedrine and also to deaminate its N-demethylated product, phenylpropanolamine.

Ephedrine is one of the sympathomimetic amines. These are a wide variety of amines structurally related to noradrenaline and adrenaline. Barger and Dale (1910) showed that these amines, in addition, have biological actions similar to adrenaline and noradrenaline. A number of years later Tainter and Chang (1927) and Tainter (1929) demonstrated that the actions of two sympathomimetic amines, tyramine and ephedrine, were antagonized by doses of cocaine which potentiated the effects of adrenaline (Fröhlich and Loewi, 1910). Thus the "cocaine paradox" originated.

Although there have been many theories to explain the action of cocaine (Furchgott, 1955), the most popular hypothesis is that cocaine acts by inhibiting the normally rapid inactivation of catecholamines by nerve uptake (Macmillan, 1959; Whitby, Hertting and Axelrod, 1960; Muscholl,1961). Cocaine has been shown to inhibit the uptake of many amines structurally related to noradrenaline; these include α-methylnoradrenaline (Hillarp and Malmfors, 1964; Muscholl and Weber, 1965), adrenaline (Iversen, 1965a; Hardman and Mayer, 1965), metaraminol

(Carlsson and Waldeck, 1965) and α-methyltyramine (Iversen, 1966).

The observation that cocaine can potentiate the action of adrenaline and antagonize that of tyramine was confirmed on isolated tissues by Burn and Tainter (1931). These workers also observed an effect which paralleled cocaine after chronic sympathetic denervation. Burn (1932) confirmed this observation when he abolished the vasoconstrictor effect of tyramine and ephedrine in the denervated cat foreleg and was the first to suggest that the action of these amines might depend upon the integrity of the adrenergic nerve endings rather than upon their direct action on adrenergic receptors. Many workers have generally classified sympathomimetic amines into one of three groups; direct acting, indirect acting, or mixed acting (Fleckenstein and Bass, 1953; Fleckenstein and Burn, 1953; Fleckenstein and Stockle, 1955; Povalski and Goldsmith, 1959; Maxwell *et al.*, 1959; 1960; Marley, 1962; Trendelenburg *et al.*, 1962a,b, 1963). Trendelenburg (1963, 1972) suggested that sympathomimetic amines possess both direct and indirect effects in varying proportions but this ratio may vary from organ to organ and also from species to species. A clear cut distinction among these, therefore, is not possible. Direct acting sympathomimetic amines are those which interact with α and/or β receptors of an effector organ whereas the indirectly acting amines act only through the liberation of the stored transmitter substance, noradrenaline. The mixed acting amines exert both direct and indirect effects. The prototype for the direct acting class is noradrenaline, for the indirect class it is tyramine, and for the mixed acting class it is ephedrine.

Ephedrine is generally classified as a mixed acting amine since both direct and indirect effects are found to be major components

in many tissues as well as species. This holds true in the nictitating membrane, blood pressure and heart rate effects in the cat (Trendelenburg *et al.*, 1962a), the dog (Maxwell *et al.*, 1959; Patil *et al.*, 1965) and guinea pig atria (Trendelenburg, 1963).

Ephedrine and many other amines structurally related to noradrenaline such as adrenaline, amphetamine, synephrine, and tyramine inhibit the uptake of noradrenaline into sympathetically innervated tissues (Axelrod and Tomchik, 1960; Axelrod, Whitby, and Hertting, 1961; Dengler, Spiegel and Titus, 1961a; Hertting *et al.*, 1961b; Axelrod, Hertting and Potter, 1962; Burgen and Iversen, 1965). It is not clearly established yet whether the amines cause inhibition of noradrenaline uptake by acting as competitive substrates for the uptake system or whether these cause inhibition of the uptake system without being themselves transported into the tissues. Adrenaline, α-methyltyramine, α-methylnoradrenaline, metaraminol, tyramine and octopamine are several sympathomimetic amines which act as alternate substrates for the noradrenaline uptake process (Shore *et al.*, 1964; Iversen, 1965a, 1966; Lindmar and Muscholl, 1965; Muscholl and Weber, 1965; Carlsson and Waldeck, 1965; Ross and Rengi, 1966a; Ross *et al.*, 1968). All of these possess at least one phenolic hydroxyl group.

Ephedrine and other amines lacking a phenolic hydroxyl group such as amphetamine, β-phenethylamine, phenylpropanolamine, and phenylethanolamine are other structural analogues of noradrenaline which appear to be accumulated in tissues in a manner different from noradrenaline (Ross and Renyi, 1966a,b, 1971; Thoenen *et al.*, 1968; Ross *et al.*, 1968; Jacquot *et al.*, 1969). In all of these cases the most

notable difference was that neither cocaine nor noradrenaline antagonized the accumulation of those amines. As a result several workers concluded that those sympathomimetic amines lacking a phenolic hydroxyl group are not transported into the adrenergic neurone by the noradrenaline uptake system (Ross and Renyi, 1966 a, b, 1971; Ross *et al.*, 1968; Jacquot *et al.*, 1969). Yet the indirect effects of ephedrine, amphetamine and phenylpropanolamine are antagonized by cocaine (Fleckenstein and Stockle, 1955; Trendelenburg *et al.*, 1962b).

Trendelenburg (1972) has suggested that this contradiction may be resolved in that the high lipophilic properties of these sympathomimetic amines lacking a phenolic hydroxyl group enables them to penetrate any cell membrane easily. The accumulation of these lipid soluble amines may occur largely in extraneuronal tissue, thus masking a small cocaine sensitive uptake. Secondly, the indirect or the noradrenaline-releasing effect may depend upon a higher rate of uptake which is achieved only with the help of the noradrenaline uptake mechanism. Inhibition of the neuronal uptake process by cocaine may slow the rate of uptake of a lipid-soluble amine enough to prevent the build-up of the high concentrations of the amine in the immediate vicinity of the neuronal vesicles needed for the release of noradrenaline into the extracellular space.

Thoenen *et al.* (1968) alternately suggested that the lipid soluble amines enter the adrenergic neurones by the same uptake process utilized by noradrenaline. Accumulation of these amines into nerve endings, however, would not occur because the lipophilic properties of these amines would enable them to diffuse rapidly and passively out of

the neurone. In this way the cocaine-sensitive uptake would not be seen.

One other possibility by which cocaine may inhibit the indirect actions of these sympathomimetic amines was suggested by Paton (1974). Because of the lipophilic properties of these amines, they can diffuse passively across the plasma membrane of adrenergic neurones. The noradrenaline which the amines subsequently displace from the intraneuronal storage vesicles effluxes from the neurone by a carrier-mediated process. Cocaine inhibits this efflux process and consequently antagonizes the indirect sympathomimetic responses of these amines.

The question that remains is: How is ephedrine which is a lipid soluble amine transported by tissues? Is the amine transported passively as suggested by Ross and Renyi (1966a,b, 1971) and Jacquot *et al.* (1969)? Since Jacquot *et al.* (1969) showed that ephedrine is taken up against a concentration gradient, then binding must follow uptake of the amine by the tissues if transport is passive. Alternately, is active transport involved? Is there any evidence that ephedrine is accumulated via the noradrenaline carrier in adrenergic nerve terminals as suggested by Trendelenburg (1972) where a large extraneuronal accumulation of the lipid soluble amine may mask a small neuronal uptake?

II. LITERATURE REVIEW

(A) General

Burn (1932) first suggested that catecholamines might be taken up into tissue binding sites. Evidence that heart tissue takes up noradrenaline and adrenaline was first demonstrated by Raab and Gigee (1955). However, this observation depended upon the administration of large doses of the amine *in vivo*. It was not until sensitive assay techniques became available that similar studies could be undertaken using physiological concentrations of the amines. Axelrod, Weil-Malherbe and Tomchik (1961) using tritiated noradrenaline and adrenaline, Muscholl (1960, 1961) using sensitive bioassay and fluorometric assay techniques and Strömblad and Nickerson (1961) using a fluorometric assay technique demonstrated an accumulation of noradrenaline in a variety of tissues. These authors suggested that this uptake might represent an important mechanism for the inactivation of noradrenaline.

Several findings suggested that the uptake of noradrenaline occurred mainly in sympathetic nerves. First, tissues which had the ability to accumulate exogenous radioactive noradrenaline lost this if the sympathetic nerves were destroyed by surgery (Hertting *et al.*, 1961a; Strömblad and Nickerson, 1961; Fischer *et al.*, 1965). Secondly, radioactive noradrenaline could be released if the intact sympathetic nerves were stimulated (Hertting and Axelrod, 1961). In the denervated tissues, a small uptake of noradrenaline still remained (Strömblad and Nickerson, 1961; Fisher *et al.*, 1965). Fischer *et al.* (1965) suggested that this residual uptake represented an extraneuronal uptake.

Iversen (1963, 1965b) described the existence in the isolated rat heart of two distinct mechanisms responsible for the uptake of noradrenaline and related sympathomimetic amines from the extracellular fluid. The first system, $Uptake_1$ or neuronal uptake is an uptake process by which the sympathomimetic amine is transported from the extracellular space across the axonal membrane of adrenergic nerves. A second mechanism known as $Uptake_2$ or extraneuronal uptake transports amines across the membranes of smooth muscle and various other post-synaptic cells. At low extracellular noradrenaline concentrations, uptake is mediated largely by the neuronal $Uptake_1$ system. $Uptake_2$, on the other hand, has a much lower affinity for noradrenaline but a very much higher capacity than $Uptake_1$. It becomes dominant at high amine concentrations. Both uptake processes are saturable. $Uptake_2$ is not normally detectable whenever the concentration of the sympathomimetic amine in the medium is low because the accumulated amine is rapidly metabolized by the actions of both monoamine oxidase and catechol O-methyltransferase (Lightman and Iversen, 1969).

The two uptake systems can be distinguished from each other on the basis of their susceptability to various inhibitors and in their relative affinities for the amines. Iversen (1964) and Burgen and Iversen (1965) systematically investigated the inhibition of noradrenaline uptake by a wide variety of sympathomimetic amines into the rat heart. All sympathomimetic amines in this study inhibited noradrenaline uptake to some degree. Amphetamine, dopamine and particularly metaraminol were among the most powerful $Uptake_1$ inhibitors. Metanephrine, normetanephrine and isoproterenol were weakly effective. The structure-

activity relationships of the noradrenaline neuronal uptake site in the rat heart found in this study can be briefly summarized as follows: β-hydroxylation, N-substitution or O-methylation decreased the affinity for the uptake site but a phenolic hydroxyl group in position 3 or 4 and α-methylation increased affinity. In the same study several of these amines were also tested as $Uptake_2$ inhibitors. The structure-activity relationships were almost the converse of those found for $Uptake_1$. N-substitution, β-hydroxylation and particularly O-methylation increased the affinity for the $Uptake_2$ site and phenolic hydroxyl groups and α-methylation decreased the affinity for the $Uptake_2$ site. Thus metaraminol and dopamine had little or no effect as $Uptake_2$ inhibitors whereas metanephrine and normetanephrine were the most powerful studied.

Many drugs other than close structural analogues of noradrenaline inhibit $Uptake_1$ (Iversen, 1965c, 1967). Tricyclic antidepressants such as imipramine, amitriptyline and desipramine are among the most potent and specific $Uptake_1$ inhibitors. Desipramine is the desmethyl derivative of imipramine and first described by Titus and Spiegel (1962) as a noradrenaline uptake inhibitor is the most potent of all known $Uptake_1$ inhibitors. A wide variety of drugs known predominantly for their other pharmacological activities also powerfully inhibit $Uptake_1$. These include the local anesthetic drug, cocaine, which serves as the classical $Uptake_1$ inhibitor; the adrenergic receptor blocking drugs, phenoxybenzamine, chlorpromazine and dichloroisoprenaline; the adrenergic neurone blocking drugs, bretylium and guanethidine; and some monoamine oxidase inhibitors, tranylcypromine, harmine and phenelzine

(Hertting *et al.*, 1962; Iversen, 1965c).

Normetanephrine, metanephrine (Burgen and Iversen, 1965) and phenoxybenzamine (Lightman and Iversen, 1969) are powerful $Uptake_2$ blockers but lack specificity. These compounds when administered at concentrations which inhibit extraneuronal uptake of noradrenaline will also cause a substantial depression of $Uptake_1$. Subsequent investigations of other β-haloalkylamines revealed that all can act as $Uptake_2$ inhibitors (Iversen *et al.*, 1972). Two of these, SKF-550 and SKF-625A were found to be much more potent and selective inhibitors of extraneuronal uptake than phenoxybenzamine. Steroids such as 17-β-estradiol, corticosterone, testosterone and progesterone are another group of compounds which can potently inhibit the uptake of noradrenaline by the $Uptake_2$ mechanism (Iversen and Salt, 1970; Salt, 1972). 17-β-estradiol and corticosterone were found to be the most potent of the group. Except for 17-β-estradiol, which also moderately inhibits $Uptake_1$, the steroids are selective inhibitors of $Uptake_2$ (Salt, 1972). Clonidine, an antihypertensive drug, is a moderately potent but a highly selective inhibitor of extraneuronal uptake (Salt, 1972).

Summary

Noradrenaline and related amines can be taken up into tissues and this by two different uptake mechanisms. Their differences can be briefly summarized:

1. Uptake of noradrenaline occurs mainly by sympathetic nerves since denervation diminishes uptake and radioactive noradrenaline can be released from sympathetic nerves

upon stimulation.

2. Iversen described the existence of two different uptake systems. Uptake$_1$, or neuronal uptake, operates at low extracellular amine concentration. Uptake$_2$, or extraneuronal uptake, operates at high extracellular amine concentration and has a lower affinity but a higher capacity for the amine. Uptake$_2$ is not normally detectable at low amine concentrations in the medium.
3. Amines exhibited the following structure-activity relationships for the noradrenaline uptake site in nerves. β-hydroxylation, N-substitution, or O-methylation decreases affinity but phenolic hydroxyl groups and α-methylation increases affinity. For Uptake$_2$ the structure-activity relationships are almost the converse.
4. Cocaine and tricyclic antidepressants are powerful and specific neuronal uptake inhibitors. SKF-550, SKF-625A and steroids are specific and potent extraneuronal uptake inhibitors.

(B) Kinetics

The uptake of noradrenaline proceeds against a concentration gradient. Brain or heart slices of the cat accumulated noradrenaline to levels five times those of the medium (Dengler *et al.*, 1961b). In the isolated rat heart perfused with a low concentration of noradrenaline in the incubation medium, the accumulation of noradrenaline by the tissue exceeded the levels in the perfusing solution by 30 times or more (Iversen, 1963; Lindmar and Muscholl, 1964). Since the sympathetic nerves occupy a very small fraction of the total weight of the heart, it is possible that the actual concentration ratio between exogenous noradrenaline accumulated in the adrenergic nerve terminals and the external medium is very high and perhaps exceeds 10,000:1.

Dengler et al. (1961b, 1962) found that the uptake of noradrenaline by cat heart and brain slices saturated as the concentration of noradrenaline in the incubation medium was increased. On the basis of this and other evidence, these authors were the first to suggest that the uptake of noradrenaline involved active transport. However, initial rates of uptake were not measured in these experiments. Iversen (1963) who studied the initial rates of uptake in detail confirmed the suggestion of Dengler *et al.* (1962) that the process obeys saturation kinetics of the Michealis-Menten type.

(C) *Stereochemical Specificity of Transport*

The noradrenaline uptake system in adrenergic nerves of the rat heart exhibits stereochemical specificity. Iversen (1963) found that the rate of uptake by the isolated rat heart of the physiologically occurring (-)-isomer of noradrenaline was several times more rapid than that of the (+)-isomer when perfused with various concentrations of (-) and (+)-noradrenaline. The same conclusion was reached independently by Maickel *et al.* (1963) who injected tritiated (±)-noradrenaline into rats and subsequently showed that there was 10 times more (-)-noradrenaline than (+)-noradrenaline in the tissue a few minutes later. However, Kopin and Bridgers (1963) reported that there was no difference in the initial uptake of either optical isomer of noradrenaline by the rat heart. Crout (1964) reported an equal uptake of either stereoisomer in the guinea pig heart. There was also very little difference in the accumulation of either stereoisomer of adrenaline in the mouse heart (Anden, 1964). Also noradrenaline uptake by the rabbit heart lacked stereochemical specificity (Draskoczy and Trendelenburg, 1968). The demonstration of a preferential accumulation of the (-)-isomer of noradrenaline rather than the (+)-isomer may be dependent upon the species of animals. Alternately it may depend on the use of small doses of the amine, since Iversen (1963) showed that the rate of uptake of (+)-noradrenaline was equal or greater than the rate of uptake of (-)-noradrenaline when high amine concentrations were used.

(D) Temperature Dependence

The transport of amines into adrenergic neurones was found to be temperature sensitive. The Q_{10} for the uptake of either noradrenaline or adrenaline by the rat uterus was found to be 2.2 indicating the process is not purely physical (Green and Miller, 1966). The temperature coefficient characteristic of a diffusion process would be about 1.2. The transport of the amines are markedly reduced when the temperature of the incubation medium was reduced to 0°C (Dengler *et al.*, 1962; Green and Miller, 1966) or preincubation at 55°C for 10 minutes (Dengler *et al.*, 1962).

(E) Effect of Metabolic Inhibition

Accumulation of noradrenaline by sympathetic nerves occurs in two steps. First there is the transport of the amine across the adrenergic neuronal membrane and secondly its incorporation into storage granules. Pretreatment with reserpine does not interfere with the transport of the amine into the neurone but does inhibit its subsequent storage (Lindmar and Muscholl, 1964; Iversen, 1967). Consequently, in reserpine-treated tissue only neuronal transport is measured. The transport of noradrenaline across the neuronal membrane in reserpine pretreated tissues has been shown to be energy dependent (Wakade and Furchgott, 1968; Paton, 1972).

Glycolysis of exogenous carbohydrates alone can provide the energy necessary to maintain the uptake of noradrenaline by rabbit or guinea pig atria since prolonged anoxia or 2,4-dinitrophenol (DNP) did

not prevent the process provided D-glucose was present in the incubation medium (Wakade and Furchgott, 1968; Paton, 1972). DNP uncouples oxidative phosphorylation and therefore should deprive the tissue of energy resulting from the mitochondrial oxidation of endogenous substrates. This would leave glycolysis as the only significant source of energy.

The neuronal uptake of amines is not exclusively dependent upon carbohydrate metabolism either. The energy required can also be supplied by oxidation of noncarbohydrate endogenous substances. The evidence for this is that prolonged glucose deprivation which most likely depletes any glycogen reserve of the adrenergic nerve terminals or the addition to the medium of the glycolytic inhibitors, iodoacetate or 2-deoxy-D-glucose, does not diminish the uptake of noradrenaline if the tissues are oxygenated (Wakade and Furchgott, 1968; Paton, 1972).

The accumulation of noradrenaline in atrial preparations is inhibited only when both glycolysis and oxidation are inhibited simultaneously (Wakade and Furchgott, 1968; Paton, 1972). Thus the energy supplied by either glycolysis of exogenous carbohydrates or oxidation of noncarbohydrate substrates can maintain the neuronal uptake of noradrenaline and related amines.

The uptake of noradrenaline in the rat uterus, vas deferens and iris was also found to be decreased when both glycolysis and oxidation were inhibited simultaneously (Hamberger, 1967; Paton, 1968). When only either oxidation or glycolysis was inhibited, the uptake of noradrenaline was prevented in cat heart slices (Dengler *et al.*, 1962), rat uterine horns (Green and Miller, 1966), and embryonic chick heart (Ignarro and Shideman, 1968). Tissue or species differences may account

for these different results. The difference in time of exposure to and concentration of inhibitors used may be factors also.

(F) *Na^+ and K^+ Requirements for Transport*

Sodium ions are an absolute requirement in the external medium for the uptake and storage of noradrenaline and other related amines such as metaraminol by sympathetic nerve endings in the rat heart (Iversen and Kravitz,1966; Gillis and Paton, 1967; Bogdanski and Brodie, 1969), in the rabbit heart (Paton, 1971) and in the rat brain synaptosomes (Bogdanski *et al.*, 1968; Colburn *et al.*, 1968; Tissari *et al.*, 1969). The stimulatory effects of Na^+ are competitively inhibited by high K^+ (Bogdanski and Brodie, 1969). The action of Na^+ on the uptake of the amines is specific for it cannot be replaced by electrolytes such as Li^+, K^+, Rb^+, Cs^+ or $choline^+$ nor by non-electrolytes such as sucrose (Bogdanski and Brodie, 1966; Paton, 1971).

Potassium ions in low concentration are needed in the incubation medium for an optimal effect in the transport of noradrenaline and other amines into adrenergic nerve terminals (Gillis and Paton, 1967; Colburn *et al.*, 1968; Paton, 1968; Bogdanski and Brodie, 1969; Sugrue and Shore, 1969). Kirpekar and Wakade (1968), however, reported that K^+ was not needed for the uptake of noradrenaline by the perfused cat spleen. A failure to adequately deplete the tissue from K^+ before determining noradrenaline uptake may have resulted in this finding. The addition of K^+ to K^+-depleted heart slices restored amine transport completely (Sugrue and Shore, 1969; Paton, 1971).

(G) Effect of Oubain

The cardiac glycosides such as oubain are known to specifically inhibit the active movements of Na^+ and K^+ across cell membranes by inhibiting the Na^+-K^+ activated adenosine triphosphatase (membrane ATPase) (Glynn, 1964; Skou, 1965). Cardiac glycosides inhibit the active transport of a wide variety of substances in a large number of tissues and in many different species (Wolff, 1960; Parrish and Kipnis, 1964; Berndt and Beechwood, 1965; Csaky and Hara, 1965; Pletscher *et al.*, 1967).

Dengler *et al.* (1961b) first showed that oubain inhibited the accumulation of noradrenaline by brain and heart slices. That oubain exerted this effect by inhibiting the transport of noradrenaline across the neuronal membrane rather than by acting on storage granules was first suggested by Carlsson *et al.* (1963). Later it was shown that oubain blocked the transport of noradrenaline and metaraminol across the neuronal membrane in rabbit and rat heart slices (Giachetti and Shore, 1966) and by rat brain synaptosomes (Tissari *et al.*, 1969). Oubain exerts this effect by acting as a non-competitive inhibitor (Berti and Shore, 1967).

The findings that tissues take up noradrenaline and structurally related amines by a process which is inhibited by oubain and is dependent upon energy, temperature, Na^+ and K^+ suggest that membrane ATPase is involved. Bogdanski and Brodie (1969) have postulated that noradrenaline transport is analogous to that proposed by Crane (1965) and Kipnis and Parrish (1965) to account for the carrier mediated transport by tissues of sugars and amino acids. According to the Bogdanski

and Brodie model, the affinity of the carrier for the amine is dependent upon its ionic environment, being increased by Na^+. Outside the nerve cell where the Na^+ concentration is high and K^+ concentration is low, the carrier has a high affinity for the amine. The amine and Na^+ are co-transported by the carrier inside the adrenergic neurone. The energy for this is provided by the downhill movement of Na^+. Once inside the cell, the amine is released where the affinity of the carrier for the amine is lowered by the low intracellular Na^+ concentration and the high intracellular K^+ concentration. After its release from the carrier, the amine is stored in intraneuronal granules. The inward transport of the amine is continued since the inward Na^+ gradient is maintained by pumping Na^+ out of the cell by the sodium pump which is linked to the Na^+-K^+ activated ATPase.

The transport of noradrenaline is blocked by oubain or K^+-free incubation medium by inhibiting the membrane ATPase. Inhibition lowers the concentration gradient of Na^+ by raising the intracellular Na^+. According to the Bogdanski-Brodie model, ATPase plays an essential but secondary role in the transport of noradrenaline. Its primary function is to maintain the Na^+-K^+ gradient.

Several findings suggest that the inwardly directed sodium gradient or the outwardly directed K^+ gradient as suggested by the Bogdanski and Brodie model cannot be the only source of energy required for the uptake of amines by the adrenergic nerves. When the sodium pump was inhibited, an induced inward Na^+ gradient failed to cause the uptake of the amine (Tissari *et al.*, 1969; White and Keen, 1970; Paton, 1971). The uptake of noradrenaline was not determined by the intracellular Na^+ concentration (White and Keen, 1970). An induced outward Na^+

gradient failed to cause an uphill efflux of the amine (Paton, 1971). An alternate possibility is that the membrane ATPase may be necessary for amine transport because the carrier must be first phosphorylated before the translocation of the amine can occur (Tissari *et al.*, 1969; Paton, 1971).

Summary

The uptake of exogenous noradrenaline and structurally related amines may be mediated by an active transport system located in the axonal membrane of adrenergic nerves. The evidence in favor of such a process can be summarized:

1. The initial rates of noradrenaline uptake are saturable and described by Michaelis-Menten kinetics.
2. Noradrenaline uptake is temperature dependent with a temperature coefficient of 2.2.
3. Noradrenaline uptake is inhibited by metabolic inhibitors such as iodoacetate and 2,4-dinitrophenol.
4. The uptake of noradrenaline requires Na^+ and K^+. Removal of Na^+ from the external medium markedly reduced the accumulation of noradrenaline. Although high concentrations of K^+ are inhibitory, low concentrations are required to maintain normal rates of uptake.
5. Noradrenaline uptake is inhibited by oubain.

(H) Objective of Thesis

The aim of this thesis is to investigate the characteristics of the transport of ephedrine by pieces of rabbit atria. The possibility that the transport of ephedrine could occur by passive diffusion, active transport, or co-transport or even by a similar mechanism as noradrenaline will be investigated. Thus answers to the following questions were sought:

1. Is ephedrine accumulated against a concentration gradient?
2. If so, is the accumulation saturable and described by Michaelis-Menten kinetics or does it increase linearly?
3. Is the accumulation of ephedrine temperature dependent?
4. What are the effects of metabolic inhibition?
5. What are the effects of Na^+ and also its substitution with other electrolytes and non-electrolytes?
6. What is the effect on accumulation by oubain?
7. Does the accumulation of ephedrine show any structural specificity?
8. Is ephedrine transported across the adrenergic neurone in a manner similar to noradrenaline?

III. METHODS AND MATERIALS

(A) *Preparation of Tissue*

Adult male New Zealand rabbits weighing 4 to 5 lbs were killed by a blow on the neck. The hearts were rapidly removed and rinsed in Krebs solution at room temperature. After both atria had been dissected free of the ventricles and adipose tissue, they were rinsed in Krebs solution, and cut into ten approximately equal pieces, weighing 20 to 40 mg each. Atrial pieces were then placed in 25 ml Erlenmeyer flasks, containing 10 ml Krebs solution. The flasks were placed in a Dubnoff metabolic shaking incubator at 37°C for 30 min and equilibrated with 95% oxygen/5% carbon dioxide.

Tissues used for time course and kinetic experiments were placed in incubation media containing the appropriate concentration of radioactive ephedrine and incubated at 37°C for the length of time required. Tissues used in experiments involving the effect of different drugs on ephedrine accumulation were preincubated for 30 min in incubation media containing the drug.

(B) *Determination of ^{14}C Content of Tissues and Media*

At the end of the incubation period, the pieces of atria were removed with forceps, blotted, placed in preweighed liquid scintillation "Minivials" and the vials weighed. The tissues were then dissolved in NCS solubilizer (Amersham/Searle) using 0.4 ml per minivial at 37°C for at least 18 hours. After making sure the tissues were completely

dissolved, 10 µl of glacial acetic acid was added to neutralize the solution and thus to minimize chemiluminescence (Bransome, 1970). 4.5 ml of a toluene-based scintillation fluor was added. The fluor had the following composition: 2,5-diphenyloxazole (PPO), 5 g; 1,4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP), 200 mg; ethylene glycol monoethyl ether, 300 ml; and toluene, 700 ml. The total ^{14}C content was measured after the vials had been cooled to room temperature and dark adapted.

Duplicate or triplicate 0.2 ml portions of incubation media were added to minivials followed by 3 ml PCS (Amersham/Searle). The vials were dark adapted and counted for ^{14}C content.

Total ^{14}C in tissue and in incubation media were counted in a Picker Nuclear Liquid Scintillation Counter (Liquimat 110) which had a counting efficiency for ^{14}C of over 90% in aqueous samples. Samples were corrected for quench using the channels ratio method.

(C) Purity of ^{14}C-Ephedrine

Radioactive ephedrine labelled with ^{14}C in the β position was obtained from Schwarz/Mann. 100 µCi of (±)-ephedrine hydrochloride was supplied in a lyophilized form with a label claim of 20 mc/mmole. Initially the drug was dissolved in its vial in 5 ml of freshly made-up diluent consisting of 0.1 mM disodium EDTA and 1 mM HCl in water. This represented a stock solution of ^{14}C-ephedrine of 10^{-3} M. From this a ten-fold dilution was made up with the same diluent and served as the working solution.

The purity of radioactive ephedrine was examined chromato-

graphically in two different solvent systems. In the first system using thin-layer plates precoated with cellulose, aliquots of the stock and working solutions were spotted and run in a n-butanol-methanol-1 N formic acid (60:20:20) system according to Fleming and Clark (1970). The second system consisted of aliquots spotted on Whatman #1 paper and run in n-butanol-acetic acid- water (2:1:1) according to the manufacturer's system. In each case development was allowed to proceed 10 cm. After the chromatogram was dried, each cm or 1/2 cm was marked off from the origin to the solvent front. The thin layer plate was scraped off into minivials and the Whatman #1 paper was cut with scissors into 1 cm strips. Five ml of the toluene based scintillation fluor was added and total ^{14}C counted in a Picker Liquimat 110 Liquid Scintillation Counter. A second chromatogram run concurrently consisted of a solution of 10^{-1} M carrier *dl*-ephedrine.

(D) Chromatogrphic Analysis of ^{14}C-Ephedrine

The results in figure 1 showed no evidence of more than a single labelled component. The peak had an R_f value for ^{14}C-ephedrine of 0.78 and 0.88 for thin-layer and paper chromatography respectively when cochromatographed with 10^{-1} M carrier ephedrine. These values were identical to those obtained with 10^{-1} M carrier ephedrine shown in figure 2.

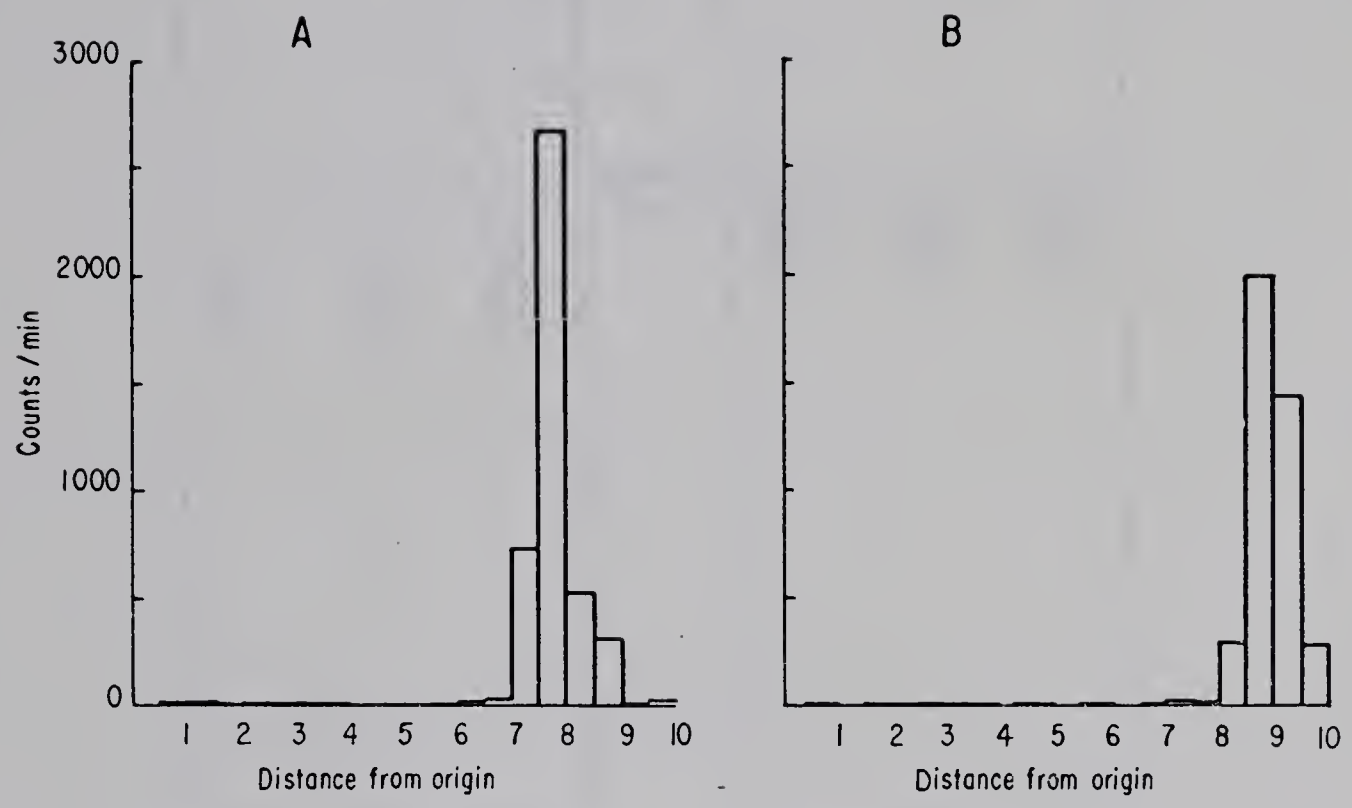


FIGURE 1. Chromatographic analyses of ^{14}C-ephedrine working solution. The data are expressed as counts per minute above background, uncorrected for quenching. The results of the thin-layer chromatogram are shown on the left and that of the paper chromatogram on the right.

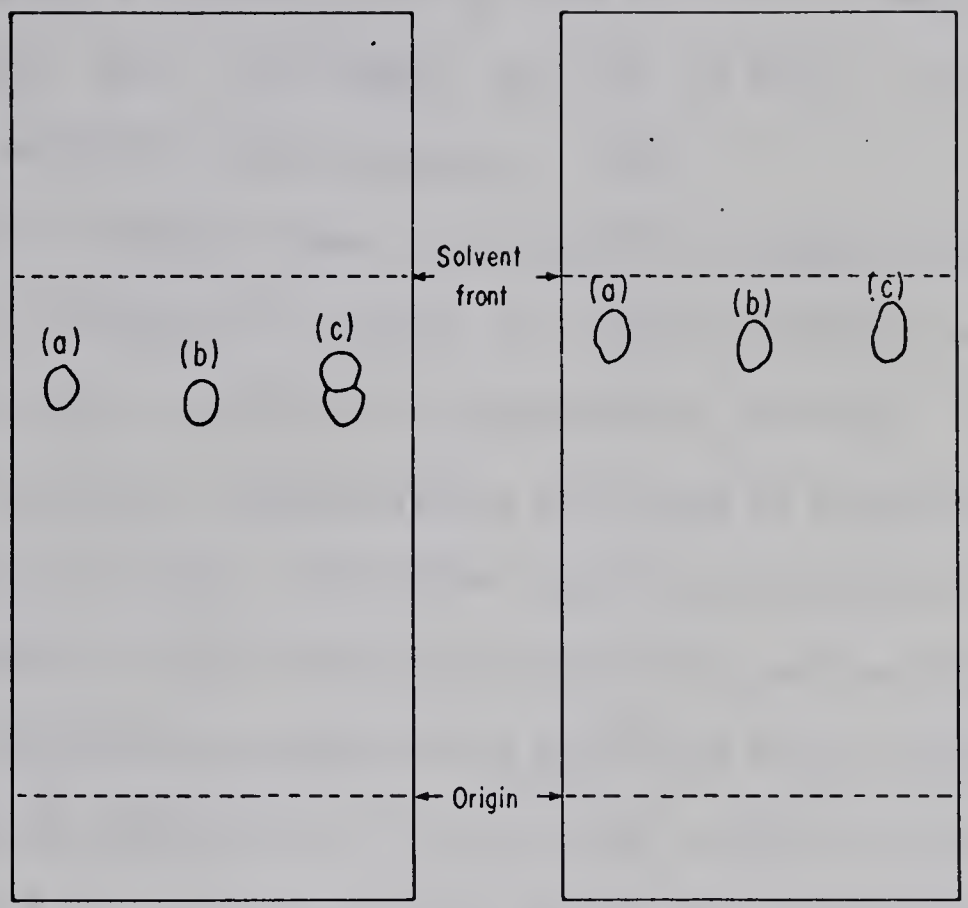


FIGURE 2. Chromatographic analysis of carrier ephedrine and phenylpropanolamine. The results of the thin-layer chromatogram are shown on the left and that of the paper chromatogram on the right. (a) represents 1 μℓ of ephedrine, 10^{-1} M; (b) represents 1 μℓ of phenylpropanolamine, 10^{-1} M; and (c) represents 1 μℓ of a mixture of the two amines.

(E) Solutions

The Krebs bicarbonate medium used had the following ionic composition (mM): NaCl, 116; $NaHCO_3$, 22; KCl, 5; $MgCl_2$, 1.2; $CaCl_2$, 1.5; D-Glucose, 10; Na_2EDTA, 0.04; ascorbate, 0.05.

KCl was omitted without substitution in potassium-free solutions. Equimolar amounts of sucrose, or 2-deoxy-D-glucose in one case, were substituted for glucose in the glucose-free solutions. Solutions containing different Na^+ concentrations were made by replacing NaCl with equimolar amounts of LiCl. Low-sodium solutions consisted of iso-osmotic replacement of NaCl with LiCl, CsCl, KCl, choline chloride and sucrose. All solutions were made using distilled water which was passed through a deionizer before use. The pH of all solutions was maintained between 7.3 and 7.5. Unless otherwise stated, all incubations were at 37°C and 95% O_2/5% CO_2 was used to equilibrate the solutions. All drugs and chemicals were weighed out daily as required.

(F) Drugs and Chemicals

The following drugs and chemicals were used and obtained from:

(±)-ephedrine.HCl	Sigma Chemical Co.
(-)-ephedrine.HCl	Regis Chemical Co.
(+)-ephedrine.HCl	Sigma Chemical Co.
(-)-ephedrine.HCl	Sigma Chemical Co.
β-phenethylamine	Sigma Chemical Co.
Tyramine.HCl	Mann Research Lab.
Dopamine.HCl	Sigma Chemical Co.

(±)-phenylpropanolamine.HCl	Sigma Chemical Co.
Metaraminol bitartrate	Merck, Sharp & Dohme
(±)- -methylnoradrenaline.HCl	Regis Chemical Co.
(±)-phenylethanolamine.	Sigma Chemical Co.
(±)-octopamine.HCl	Sigma Chemical Co.
(±)-noradrenaline.HCl	Sigma Chemical Co.
(-)-amphetamine sulfate	Smith, Kline & French
(+)-amphetamine sulfate	Smith, Kline & French
(±)-parahydroxyamphetamine.HBr	Smith, Kline & French
(±)-isoproterenol.HCl	Winthrop Lab.
Phentermine.HCl	Anca Lab
(±)-normetanephrine.HCl	Sigma Chemical Co.
Ouabain	Sigma Chemical Co.
2-deoxy-D-glucose	Aldrich Chemical Co.
Iodoacetic acid	Eastman Kodak Co.
α-dinitrophenol	Fisher Scientific Co.
Sodium azide	Fisher Scientific Co.
Cocaine-HCl	British Drug Houses
Desipramine.HCl	Geigy
Lidocaine.HCl	Astra Pharmaceuticals
Phenoxybenzamine.HCl	Smith, Kline & French
Phentolamine	Ciba
Testosterone	Sigma Chemical Co.
Estradiol	Regis Chemical Co.
6-Hydroxydopamine.HBr	Regis Chemical Co.
SKF-550	Smith, Kline & French

(G) Preparation of Denervated Tissues

Adrenergic denervation was produced using 6-hydroxydopamine. 6-Hydroxydopamine was dissolved in 1 ml freshly prepared 1% ascorbic acid in ice cold normal saline and injected immediately into the lateral ear vein. Rabbits were given 30 mg each at 9:00 hr on day 1, followed by 90 mg at 16:00 hr on day 1, and 90 mg again at 9:00 hr on day 2. They were killed at about 10:00 hr on day 3 and their hearts removed.

(H) Expression of Results

The accumulation of ^{14}C-ephedrine was expressed in one of the following ways:

(a) pmoles/gm wet weight tissue

$$= \frac{\text{disintegrations per min/gm wt tissue}}{\text{disintegrations per min/pmole } ^{14}\text{C-ephedrine}}$$

(b) T/M ratio

$$= \frac{\text{disintegrations per min/gm wt tissue}}{\text{disintegrations per min/ml incubation medium}}$$

(I) Metabolism of ^{14}C-Ephedrine in the Tissues

To investigate the possibility that ephedrine metabolites may be present, pieces of atria were incubated in media containing ^{14}C-ephedrine for 1 hour. The tissues were then removed and blotted. These were pooled and homogenized in 0.1 N HCl containing 0.1 mM Na_2EDTA in a ground-glass grinder. The homogenate was centrifuged at 0°C and the supernatant poured off and saved. The supernatant was then concentrated

over a stream of air. An aliquot of this concentrated extract was then taken for thin-layer and paper chromatography. Also aliquots of the incubation media before and after the incubation of the tissue and the supernatant before concentration by a stream of air were chromatographed. The location of ^{14}C was determined by counting successive 1/2 cm portions of the chromatograms by liquid scintillation spectrometry.

Chromatographic analysis of the concentrated tissue extract shown in figure 3 revealed only one radioactive peak which had an R_f value identical to the cold ephedrine in figure 2 and also to the ^{14}C-ephedrine working solution. The results were similar when the incubation media before and after tissue incubation and the supernatant before concentration were chromatographed. Subsequently in this study, measurements of radioactivity in the tissue will be expressed as ^{14}C-ephedrine.

Axelrod (1953) showed that in rabbits and some other animals, the N-demethylated product of ephedrine, phenylpropanolamine, was excreted. It is expected that ephedrine would be N-demethylated enzymatically in the liver but not in the heart. Figure 2 shows that ephedrine and phenylpropanolamine have almost identical R_f values in the two systems used and thus it would not be possible to detect phenylpropanolamine if it is present in significant amounts. The result is not unexpected since the two amines are very similar in structure and polarity.

(J) Statistical Analysis

Results were expressed as the mean±standard error of the mean. Significant differences between samples were determined using Student's t-test and were considered to be significant when $p<0.05$ (two-tailed test).

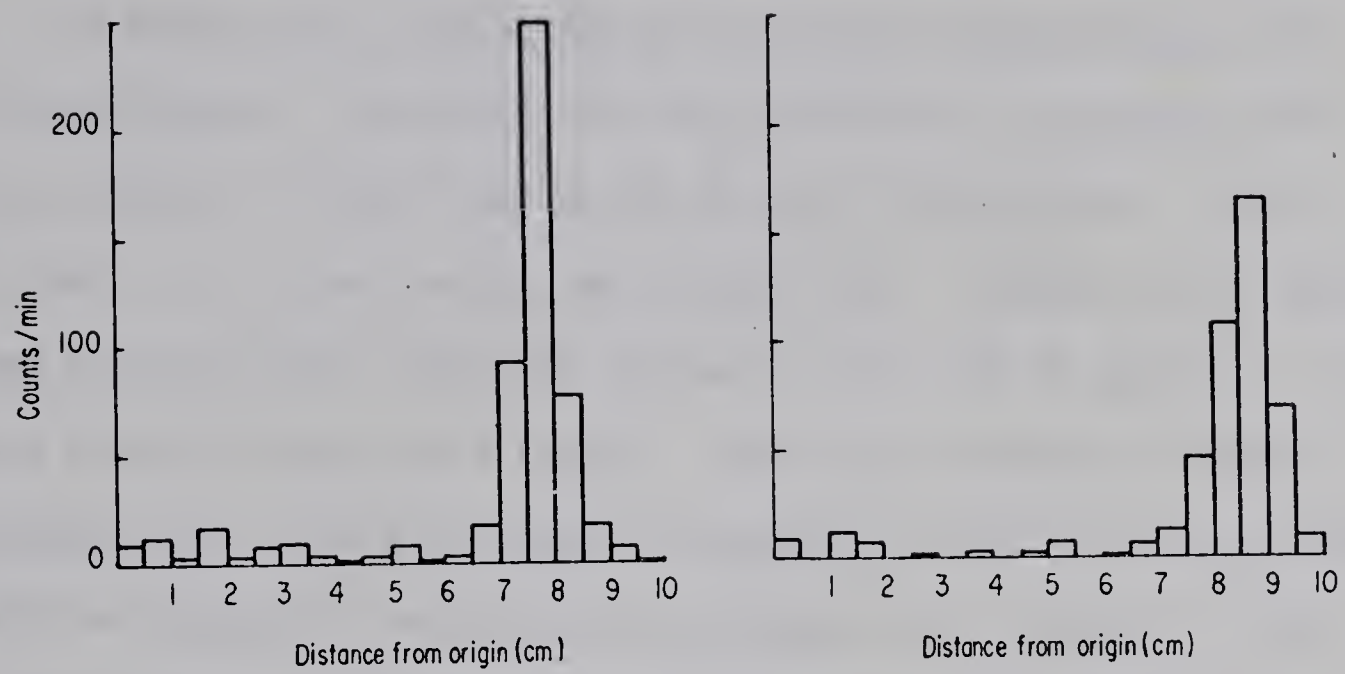


FIGURE 3. Chromatographic analysis of pooled tissue extracts. The data are expressed in counts per minute above background, uncorrected for quenching. The result of the thin-layer chromatogram is shown on the left and that of the paper chromatogram on the right.

IV. RESULTS

(A) Effect of Drugs and Conditions on Tissue Weight Loss

In this study, calculation of uptake was dependent upon the final tissue weight. The effects of the experimental procedures were therefore examined. Table 1 shows the results of this study. It can be seen that in all cases, weight was always lost. Tissues only showed shrinkage when they were incubated in low Na^+ plus 210 mM sucrose. Thus a falsely high T/M ratio would result. There was evidence of tissue swelling when the tissue was exposed to metabolic inhibitors, particularly when both the glycolytic and oxidative pathways were inhibited. This would consequently result in a falsely lower T/M. The remainder of the drugs or conditions produced little or no change in the weight of the tissue compared to the control. This study illustrates the care which must be taken in the interpretation of results because significant changes may be produced just by the shrinkage or swelling of the tissue under experimental conditions.

(B) Ephedrine Uptake

The effect of different incubation times on the uptake of ^{14}C-ephedrine (T/M ratio) is shown in figure 4. The upper curve shows the uptake of ephedrine when the concentration was 10^{-6} M and the lower curve shows the uptake when the concentration of the amine in the medium was 10^{-3} M. The two curves are similar in that accumulation was time dependent, net uptake becoming progressively slower with time. Ephedrine

TABLE 1

EFFECT OF DRUGS AND CONDITIONS ON TISSUE WEIGHT LOSS

Tissues were washed for 30 minutes, blotted and weighed and then exposed to two 30 min periods to the treatment shown. Tissues were weighed immediately thereafter. Mean ± S.E. of 6 observations.

TREATMENT	% WT. LOSS	t-TEST
Control	14.3±1.6	
27°C	16.7±2.1	NS
4°C (ice)	10.5±1.7	NS
Oubain, 10^{-4} M	12.1±2.0	NS
Phentermine, 10^{-3} M	13.7±1.3	NS
10^{-2} M	14.2±2.0	NS
Low Na^{+} + LiCl (116 mM)	14.6±1.5	NS
+ Sucrose (210 mM)	20.3±1.3	$p<0.05$
Control	17.1±1.5	
2-deoxy-D-glucose, 2 x 10^{-3} M	13.0±0.8	$p<0.05$
Iodoacetate, 10^{-3} M	16.4±2.1	NS
2,4-Dinitrophenol, 5 x 10^{-4} M	13.1±3.7	NS
Sodium Azide, 10^{-3} M	11.9±1.8	NS
2,4-Dinitrophenol, $5x10^{-4}$M + Iodoacetate, 10^{-3}M	5.1±0.5	$p<0.001$
Glucose-free	12.7±3.0	NS
+ 2-deoxy-D-glucose, 10 mM	13.4±2.4	NS
+ Iodoacetate, 10^{-3} M	8.8±1.1	$p<0.005$
+ 2,4-Dinitrophenol, $5x10^{-4}$M	11.0±3.3	NS

was initially accumulated by slices of rabbit atria very rapidly and after a short period of incubation, the amount in the tissue exceeded that in the medium. Steady-state levels were reached at about 1 hour of incubation. The two curves differ in that the T/M ratio was lower at all incubation times when the higher concentration of ephedrine was present in the incubation medium and also a shorter time of incubation was required for the tissue to concentrate ephedrine above that found in the medium when the lower concentration of the amine was used.

(C) Effect of Concentration on Ephedrine Uptake

At the end of 1 hour incubation the T/M ratio was 2.0 at the higher ephedrine concentration whereas it was 2.7 at the lower concentration (figure 4), and thus indicated that some saturation had taken place. This led to a study in which the uptake of ephedrine by rabbit atria was measured after 30 min incubation at external amine concentrations varying from 2.5×10^{-7} M to 10^{-3} M. Figure 5 shows that as the concentration of ephedrine in the incubation medium was increased, uptake (expressed as pmoles/gm wet tissue) increased progressively without an indication of a maximum being approached. It should be mentioned that although the accumulation of ephedrine does not appear to approach a maximum, it may not necessarily mean that the system is non-saturable but that it may saturate at only inaccessibly high concentrations. Since such a wide concentration range of ephedrine was used and the results in figure 4 indicated saturation, the results of figure 5 were re-expressed as concentration ratios (T/M ratios) to detect small changes. It can be seen in figure 6 that the T/M ratios slowly decreased

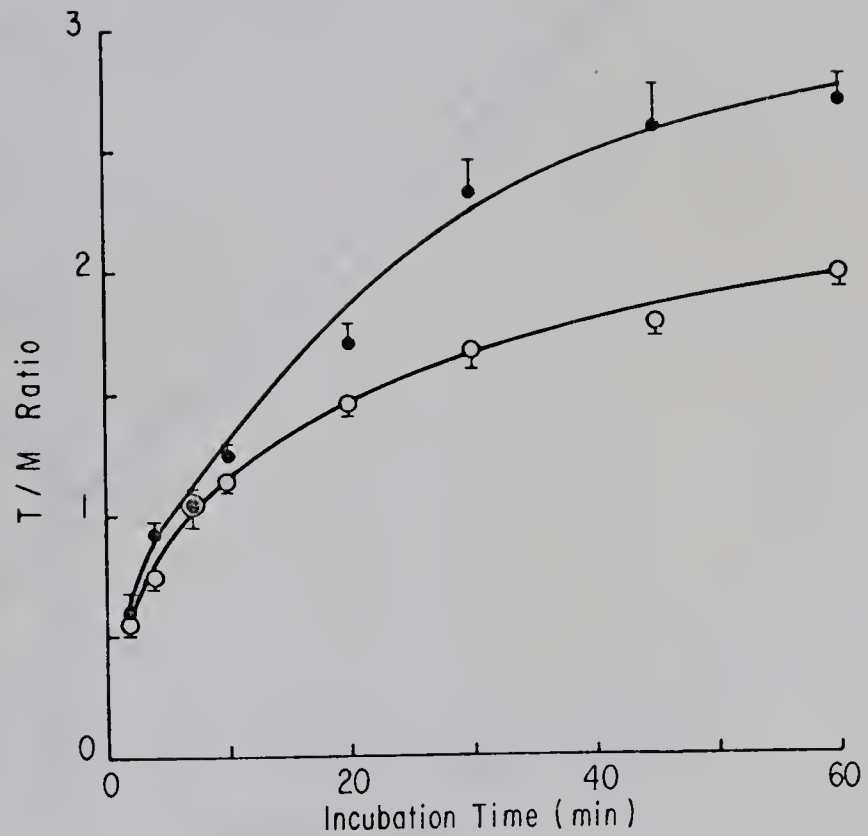


FIGURE 4. ^{14}C-ephedrine accumulation by rabbit atrial pieces. The tissue/medium (T/M) concentration ratio of ephedrine is plotted against the duration of incubation (minutes) and is shown as the mean ± S.E. ●——●, the ephedrine concentration in the medium was 10^{-6} M. o——o, 10^{-3} M ephedrine was added to 10^{-6} M labelled ephedrine as a carrier. There were 6 - 18 observations for each point.

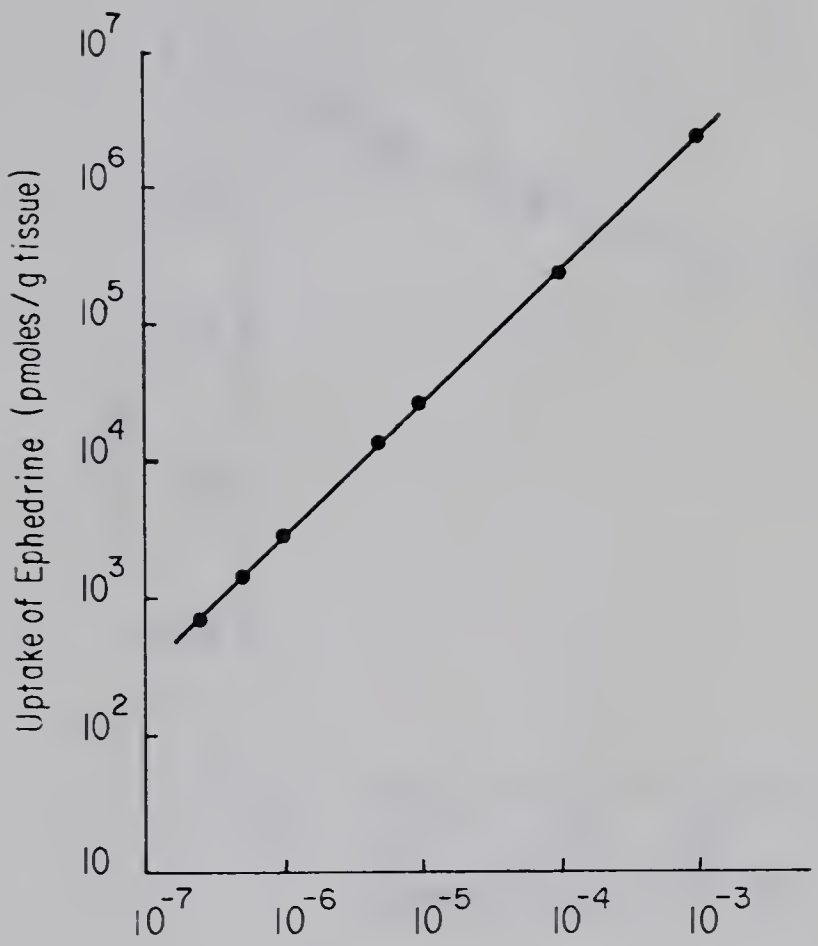


FIGURE 5. Effect of concentration on ephedrine uptake by rabbit atria. Tissues were incubated for 30 min at 37°C in Krebs solution containing concentrations of ephedrine varying from 2.5×10^{-7} to 10^{-3} M. At ephedrine concentrations greater than 5×10^{-7} M, carrier (±)-ephedrine was added to make up the desired concentration with 5×10^{-7} M labelled ephedrine. Mean values of 10 observations. S.E.s fall inside the circles.

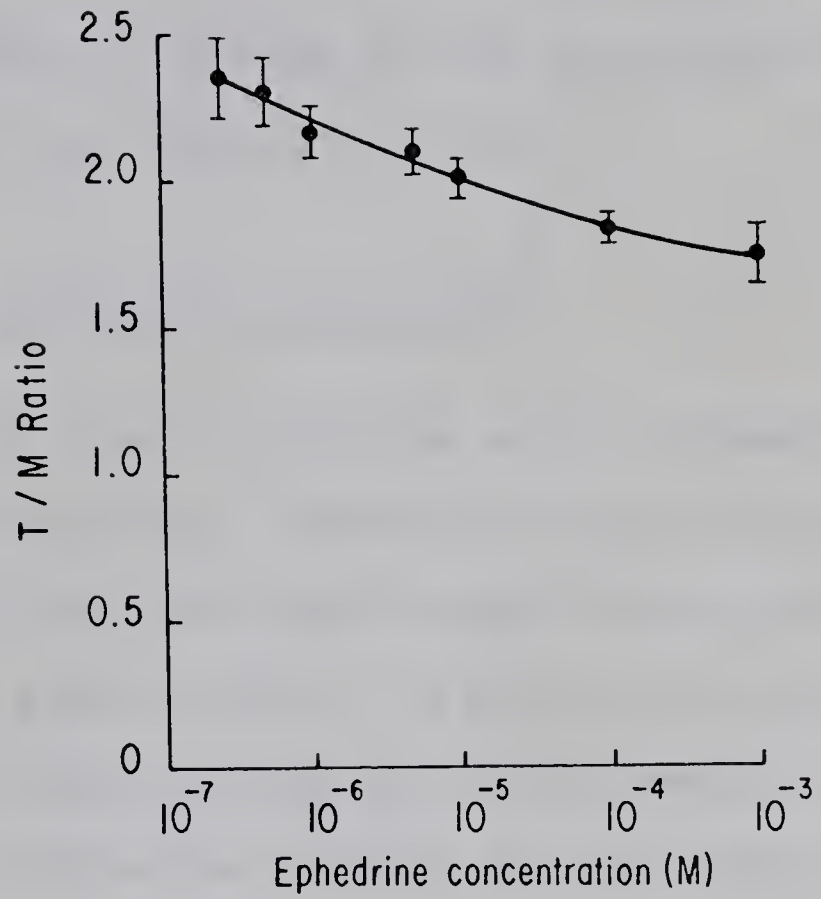


FIGURE 6. T/M ratio at different ephedrine concentrations. The tissue to medium ratio of ephedrine is plotted against the concentration of ephedrine in the medium and is shown as the mean ± S.E. At ephedrine concentrations greater than 5×10^{-7} M, carrier (±)-ephedrine was added to make up the desired concentration with 5×10^{-7} M labelled ephedrine. Incubation time was 30 min. There were 10 observations for each point.

from a value of approximately 2.35 at an ephedrine concentration of 2.5×10^{-7} M in the incubation medium to 1.7 at 10^{-3} M indicating some saturation has taken place. Because of the small amount of radioactivity found in the tissue, it was not possible to use ephedrine concentrations in the medium lower than 2.5×10^{-7} M.

(D) Initial Rates of Ephedrine Accumulation

As shown in figures 4 and 6 the uptake of ephedrine indicated a tendency towards saturation. Therefore an attempt was made to show whether this uptake can be described in terms of the classical Michealis-Menten equation for enzyme kinetics. In order to do so, the initial rates of uptake (the velocity component) was measured at varying ephedrine concentrations and at the shortest incubation time possible. Figure 7 shows that when ephedrine concentrations were varied from 2.5×10^{-7} M to 10^{-2} M, the amine accumulated in the tissue at a uniform rate without any indication of maximum velocity being approached. The rapid initial phase, therefore, cannot be described by the Michealis-Menton equation. It appears from these results to be due solely to a passive diffusion process.

(E) Effect of Various Amines on Ephedrine Accumulation

A wide variety of sympathomimetic amines are known to inhibit the uptake of noradrenaline. The effect of some of these amines on the uptake of ephedrine (T/M ratio) was studied. Table 2 shows that only normetanephrine of the amines studied failed to decrease ephedrine

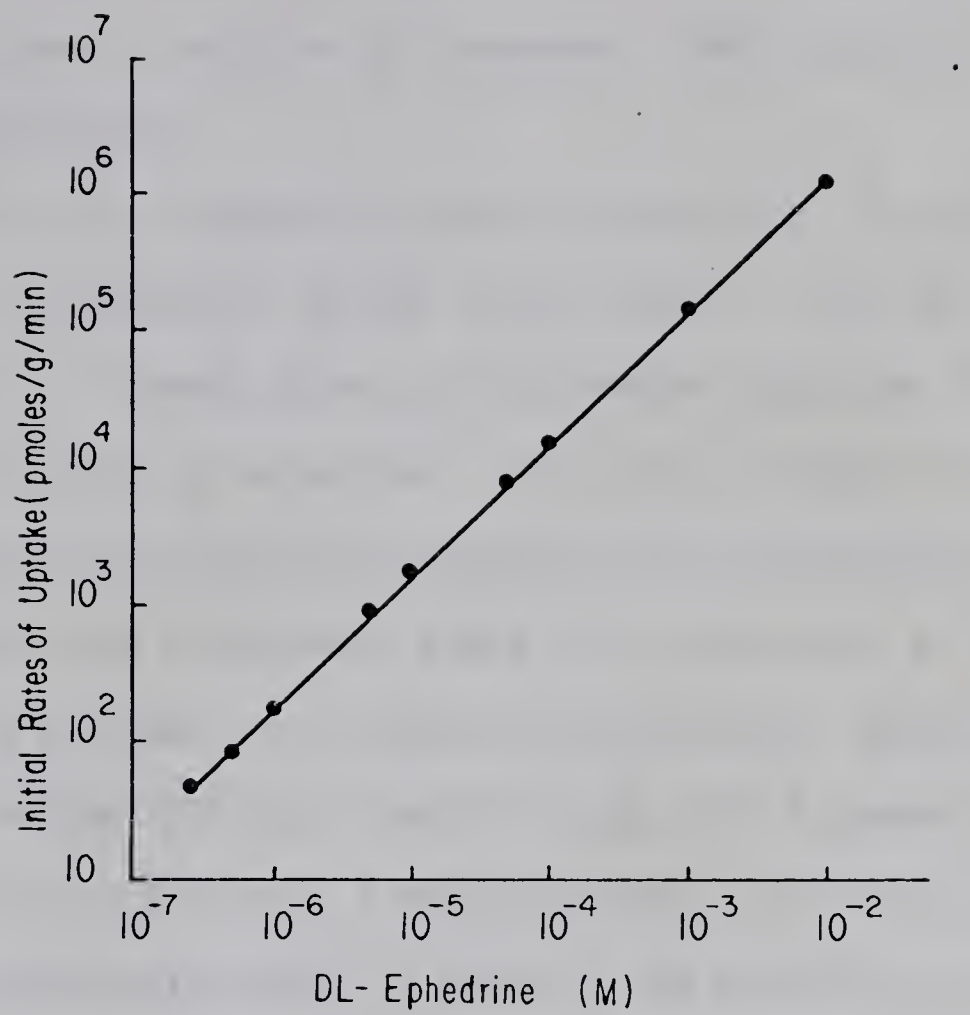


FIGURE 7. Initial rates of ephedrine accumulation. Tissues were incubated for 10 min at 37°C in Krebs solution containing concentrations of (±)-ephedrine varying from 2.5×10^{-7} M to 10^{-2} M. Mean values of 6 observations.

accumulation significantly. Phentermine caused the greatest inhibition of ephedrine uptake but even in its presence a large fraction of ephedrine still accumulated.

The effects on ephedrine accumulation by 10^{-7} M to 10^{-2} M phentermine was subsequently studied in more detail. The results are shown in figure 8. Concentrations of phentermine less than 10^{-5} M had no effect on the uptake of ephedrine. The uptake of ephedrine is only slowly decreased with increasing concentrations of phentermine in the incubation medium, and furthermore there is no indication of maximal inhibition being produced. Increasing the phentermine concentration in the incubation medium 1000-fold from 10^{-5} M to 10^{-2} M caused only a small decrease in the T/M ratio from approximately 2 to 1.3.

From the results shown in table 2, the possibility must be considered that the inhibition of ephedrine by amines may be related to the number of hydroxyl groups the amine possesses. The various amines were grouped as shown in table 3 to illustrate the effects of phenolic hydroxyl groups on ephedrine uptake. It can be seen that those amines in each group which lacked phenolic hydroxyl groups all caused the greatest inhibition of ephedrine accumulation. The amines which possess two phenolic hydroxyl groups, however, inhibited ephedrine uptake approximately to the same extent as those amines which possess one phenolic group. Because of the small amount of inhibition produced by the amines possessing 1 or 2 phenolic hydroxyl groups, it was not possible to be able to detect any other structural specificities on ephedrine transport.

Whether or not ephedrine transport exhibits stereochemical

TABLE 2

EFFECT OF VARIOUS AMINES ON EPHEDRINE ACCUMULATION

Tissues were preincubated for 30 min in normal Krebs solution containing the amines at a concentration of 10^{-3} M. 10^{-6} M ephedrine was then added for an additional 30 min. Mean ± S.E. of 10 observations.

TREATMENT	T/M	t-TEST
Control (normal Krebs)	2.08 ± 0.06	
Metaraminol	1.77 ± 0.04	$p < 0.001$
Noradrenaline	1.87 ± 0.07	$p < 0.05$
Normetanephrine	1.96 ± 0.08	NS
Isoproterenol	1.83 ± 0.08	$p < 0.02$
Phentermine	1.55 ± 0.07	$p < 0.001$

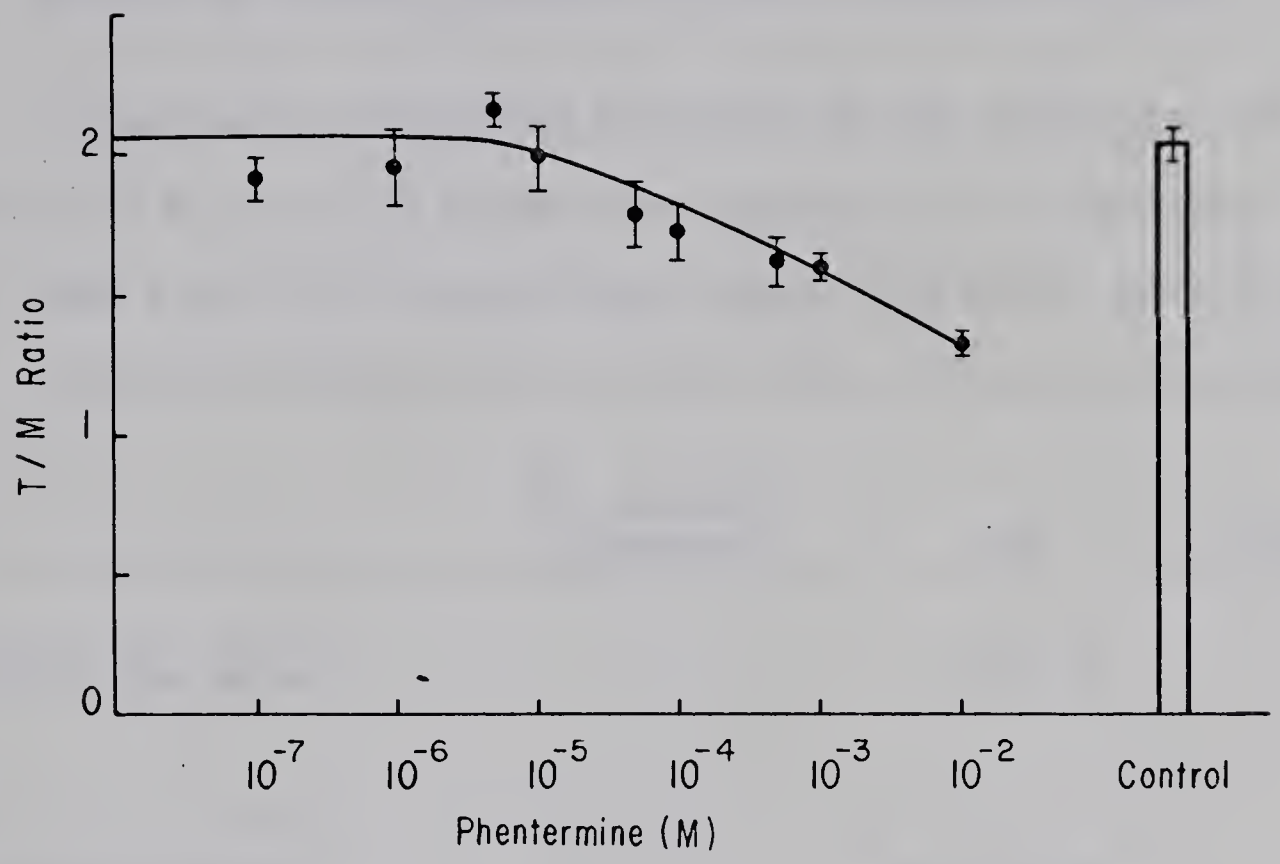


FIGURE 8. Effect of phentermine on ephedrine accumulation. Tissues were exposed to varying concentrations of phentermine for 30 min. 5×10^{-7} M ephedrine was added for an additional 30 min. Mean ± S.E. of 6 observations. The control value is shown on an histogram.

TABLE 3

EFFECTS OF PHENOLIC HYDROXYL GROUPS ON EPHEDRINE UPTAKE

Tissues were preincubated for 30 min in the presence of the amines at 10^{-3} M. 5×10^{-7} M ephedrine was added for an additional 30 min. Mean ± S.E. of 10 observations except in Group IV where there were 6. Asterisks indicate values significantly different from control.

AMINE	NO. PHENOLIC HYDROXYLS	T/M	t-TEST
Control (No amine)		2.13±0.10	
Group I			
β-phenethylamine	0	*1.63±0.04	
Tyramine	1	1.89±0.08	p<0.02
Dopamine	2	1.91±0.11	p<0.05
Group II			
Phenylpropanolamine	0	*1.67±0.07	
Metaraminol	1	1.88±0.08	NS
α-methylnoradrenaline	2	2.10±0.11	p<0.005
Group III			
Phenylethanolamine	0	*1.72±0.06	
Octopamine	1	1.87±0.07	NS
Noradrenaline	2	*1.86±0.06	NS
Group IV			
Amphetamine	0	*1.48±0.06	
p-hydroxyamphetamine	1	*1.61±0.02	NS
α-methyldopamine	2	*1.74±0.07	p<0.05
Control (No amine)		2.08±0.09	

specificity was next considered. The effect of (±)-, (-)-, (-)-ψ-, (+)-ψ-ephedrine and (+)- and (-)-amphetamine isomers on ephedrine uptake was studied. It can be seen in table 4 that the ephedrine isomers at a concentration of 10^{-3} M all significantly decreased the uptake of labelled ephedrine with approximately the same potency. Lower concentrations of ephedrine and also the amphetamine isomers, 5×10^{-5} M, were subsequently studied in an attempt to detect differences in potencies. The results in table 4, part b, show that all ephedrine and amphetamine isomers at a concentration of 5×10^{-5} M slightly decreased the uptake of labelled ephedrine but with equal potency.

(F) Inhibition of Ephedrine Uptake by Cocaine and Desipramine

The effects on ephedrine uptake of the specific uptake$_1$ inhibitors, 2×10^{-5} - 2×10^{-3} M cocaine and 10^{-6} - 10^{-4} M desipramine, were studied. Since high concentrations of cocaine and desipramine may act on the tissue as local anesthetics, the effect of 5×10^{-5} - 5×10^{-3} M lignocaine, a local anesthetic known not to inhibit uptake$_1$, was also studied. It can be seen in figure 9 that all three drugs produced a concentration dependent inhibition of ephedrine accumulation. Cocaine and desipramine inhibited ephedrine accumulation with approximately equal potency. Lignocaine was much less potent at lower concentrations and thus eliminating the possibility that the inhibitory effect is due to a local anesthetic effect. At the highest concentrations used, lignocaine inhibited ephedrine accumulation as potently as cocaine or desipramine. However, in all cases, the local anesthetic effect was small since a large fraction of ephedrine still accumulated.

TABLE 4

EFFECT OF THE ISOMERS OF EPHEDRINE AND AMPHETAMINE ON EPHEDRINE UPTAKE

Tissues were preincubated for 30 min in the presence of the amines. 5×10^{-7} M ephedrine was added for an additional 30 min. Mean ± S.E. of 10 observations in (a) and 6 in (b).

TREATMENT	T/M	t-TEST
(a) 10^{-3} M Amines		
Control (No amines)	2.35 ± 0.14	
(±)-ephedrine	1.74 ± 0.10	$p < 0.001$
(-)-ephedrine	1.63 ± 0.06	$p < 0.001$
(+)-Ψ-ephedrine	1.78 ± 0.06	$p < 0.001$
(-)-Ψ-ephedrine	1.64 ± 0.06	$p < 0.001$
(b) 5×10^{-5} M Amines		
Control	2.08 ± 0.09	
(±)-ephedrine	1.89 ± 0.06	NS
(-)-ephedrine	1.85 ± 0.08	NS
(+)-Ψ-ephedrine	1.80 ± 0.10	NS
(-)-Ψ-ephedrine	1.84 ± 0.11	NS
(-)-amphetamine	1.88 ± 0.04	NS
(+)-amphetamine	1.81 ± 0.07	$p < 0.05$

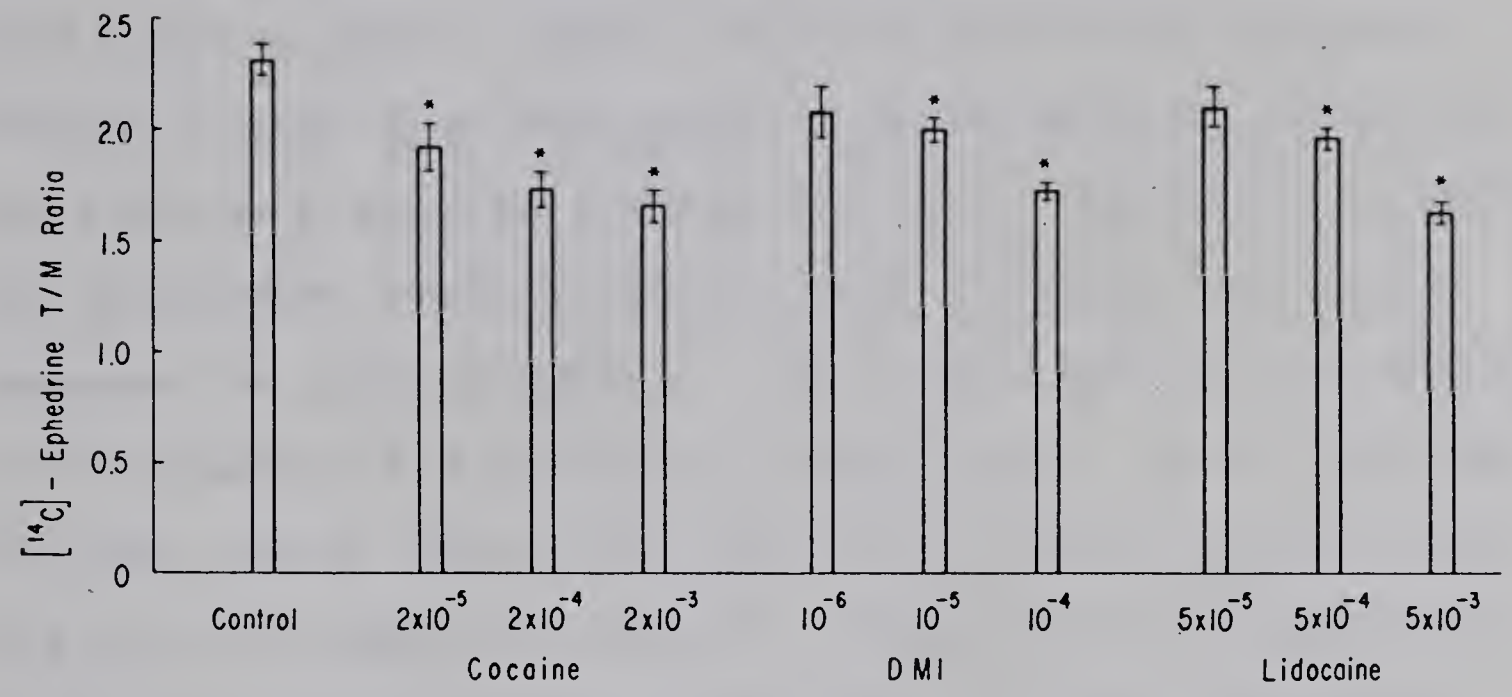


FIGURE 9. Effect of cocaine and desipramine on ephedrine accumulation. Pieces of rabbit atria were preincubated in Krebs solution in the presence of the drugs for 30 min. 5×10^{-7} M ephedrine was added for an additional 30 min and accumulation measured. Mean ± S.E. There were 10 observations for each column. Asterisks indicate values significantly different from the control which had no drugs present.

(G) The Effect of Other Drugs on Ephedrine Accumulation

The effect of a variety of drugs on the uptake of ephedrine (T/M ratio) is shown in table 5. It can be seen that the adrenergic receptor blocking drugs phentolamine, phenoxybenzamine and SKF-550 were not effective as ephedrine transport inhibitors. The steroids, estradiol, and testosterone, which are powerful $uptake_2$ inhibitors significantly decreased the uptake of ephedrine. But a large fraction of the amine was still accumulated in the presence of these steroids. On the other hand, the other steroid used, corticosterone, also a powerful $uptake_2$ blocker did not affect ephedrine accumulation. Because 100-fold concentrations of the stock solutions of the steroids were dissolved in 30% ethanol, the effects of this ethanol solution (0.3% when used) were studied as a control. As shown in table 5,0.3% ethanol did not exert any effect of its own. One other drug oxytetracycline, reported to inhibit the binding of catecholamines to collagen (Powis, 1973) was studied in this series. It too did not affect ephedrine accumulation (table 5). A combination of three drugs, cocaine, oxytetracycline and corticosterone were added simultaneously to the Krebs solution. Although this procedure decreased the uptake of ephedrine significantly, the effect as shown in table 5 was small. The cocaine control tested in this series showed that this effect could not be attributed to cocaine.

(H) Effect of Exposure to Oubain

Oubain is known to have an effect on active transport. The effect of 10^{-6}- 10^{-3} M oubain was studied to determine whether Na^{+}-K^{+}-

TABLE 5

EFFECT OF OTHER DRUGS ON EPHEDRINE ACCUMULATION

Tissues were preincubated 30 min in the treatments shown. 5×10^{-7} M ephedrine was added for an additional 30 min. Drugs were present throughout. Concentration of drugs shown in brackets. Mean ± S.E. of 6 - 8 observations.

TREATMENT	T/M	CONTROL T/M	t-TEST
Phentolamine (10^{-5} M)	1.99±0.09	2.14±0.06	NS
Phenoxybenzamine (10^{-5} M)	1.91±0.06	2.14±0.06	NS
SKF-550 (10^{-5} M)	1.96±0.17	2.13±0.10	NS
Estradiol (10µg/ml)	1.83±0.07	2.13±0.10	$p<0.05$
Testosterone (10 µg/ml)	1.82±0.07	2.13±0.10	$p<0.05$
Corticosterone (10 µg/ml)	1.99±0.10	2.13±0.10	NS
Ethanol (0.3%)	2.10±0.10	2.13±0.10	NS
Oxytetracycline (10^{-4} M)	2.10±0.09	2.13±0.10	NS
Cocaine Control (2×10^{-5} M)	1.96±0.10	2.13±0.10	NS
Oxytetracycline(10^{-4}M)+Cocaine(2×10^{-5}M)+ Corticosterone (10 µg/ml)	1.73±0.07	2.13±0.10	$p<0.01$

activated membrane ATPase was involved in the transport of ephedrine. It can be seen (figure 10) that oubain produced a small concentration dependent inhibition of uptake. Extremely high concentrations of oubain (10^{-4} - 10^{-3} M) were required in order to produce a significant inhibition of ephedrine accumulation.

(I) Effect of Na^+ and K^+ on Ephedrine Accumulation

Oubain is known to inhibit Na^+-K^+-ATPase, the enzyme involved in the active transport of sodium. This process is commonly called the Sodium Pump. Since oubain inhibited ephedrine transport, the possibility that this uptake occurs by a co-transport mechanism with sodium must be considered. The influence of sodium on ephedrine uptake therefore was studied. Figure 11 shows the relationship between the 30 min uptake of ephedrine (expressed as the T/M ratio) and the concentration of Na^+ in the incubation medium. It can be seen that sodium enhanced the uptake of ephedrine but was not an absolute requirement and that a large fraction (over 80%) of the amine was accumulated in the presence of low Na^+.

In table 6 the uptake of ephedrine in Krebs solution was compared with that in low Na^+ solution in which 116 mM NaCl was replaced iso-osmotically by one of the following: LiCl, CsCl, KCl, choline chloride and sucrose. Of these only sucrose failed to reduce uptake significantly. In all cases, however, a large fraction of ephedrine still accumulated. Sucrose caused the tissue to shrink (table 1) and this accounts for the observation.

The effect of removing K^+ from the incubation medium is shown in table 7. During the preincubation period, the K^+-free medium was

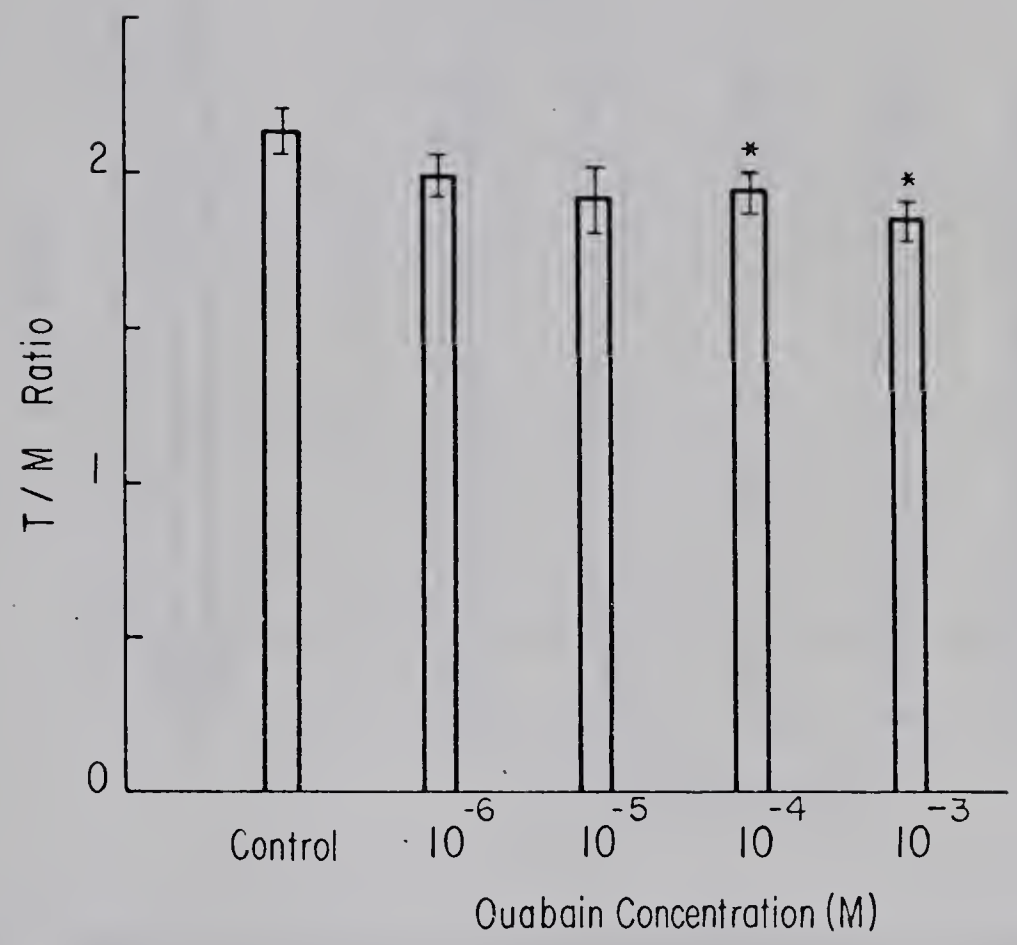


FIGURE 10. Effect of oubain on ephedrine accumulation. Pieces of rabbit atria were exposed to varying concentrations of oubain for 30 min. Then after a further 30 min of incubation with 5×10^{-7} M ^{14}C-ephedrine, accumulation was determined. Mean ± S.E. of 9 - 10 observations. The columns marked with an asterisk differed significantly from the control incubated in media containing no oubain.

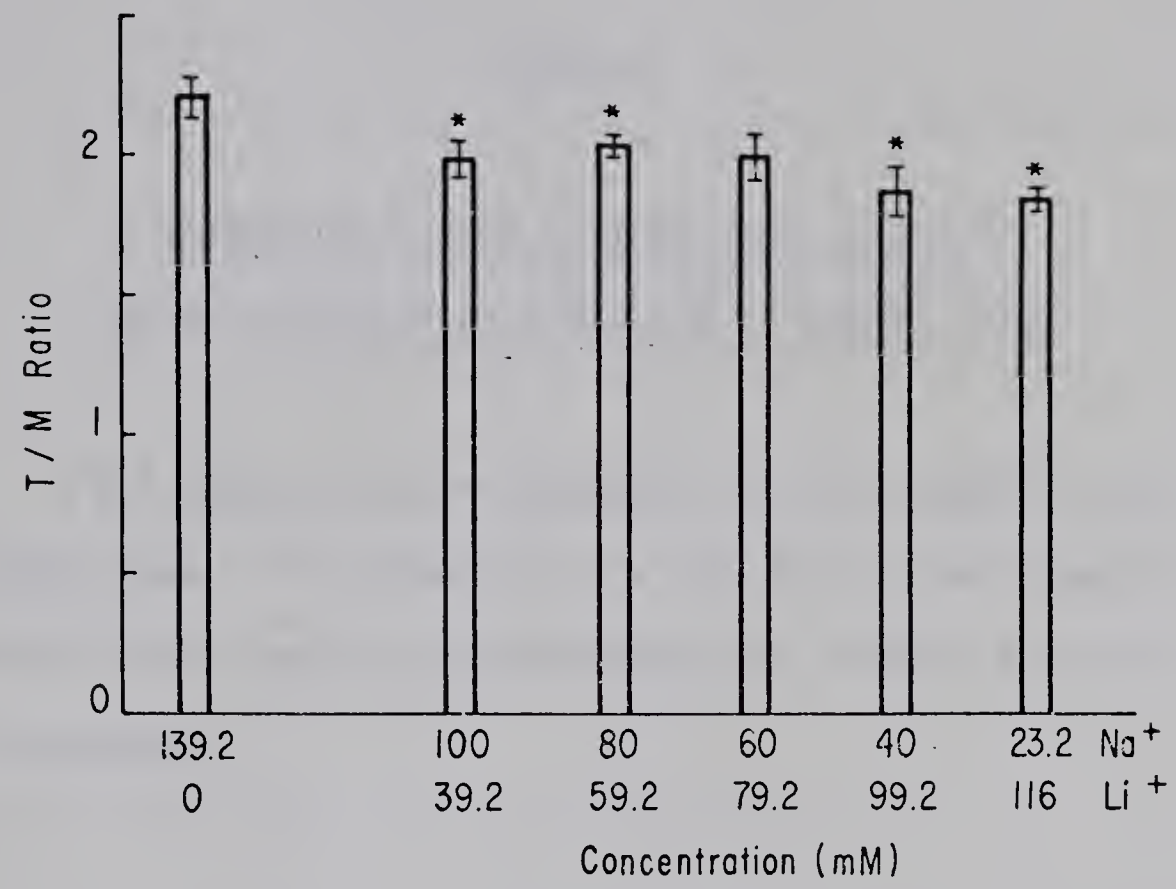


FIGURE 11. Effect of varying sodium concentrations on the transport of ^{14}C-ephedrine. Pieces were exposed for 30 min to varying sodium chloride concentrations which were replaced iso-osmotically by lithium chloride. Accumulation was determined after 30 min incubation at 37°C with 5×10^{-7} M ephedrine and is shown as the mean ± S.E. There were 10 observations for each point. The values marked with an asterisk differed significantly from those obtained in normal Krebs solution containing 139.2 mM Na^+.

TABLE 6

INABILITY OF IONS TO SUBSTITUTE FOR Na^+

IN THE TRANSPORT OF EPHEDRINE BY RABBIT ATRIA

All pieces were preincubated for 30 minutes in the solutions shown. ^{14}C-ephedrine, 5×10^{-7} M was then added for a further 30 min and accumulation measured. Mean ± S.E. of 10 observations.

INCUBATION MEDIA	T/M	t-TEST
Normal Krebs	2.20 ± 0.07	
Low Na^+ Krebs Containing:		
LiCl	1.84 ± 0.04	$p < 0.005$
CsCl	1.88 ± 0.04	$p < 0.010$
KCl	1.76 ± 0.03	$p < 0.005$
Choline Cl	1.84 ± 0.03	$p < 0.005$
Sucrose	2.16 ± 0.09	NS

changed every 10 min to ensure the removal of as much K^+ from the tissue as possible. It can be seen that the removal of K^+ did not alter the uptake of ephedrine. The addition of 10^{-4} M oubain to K^+-free medium decreased the accumulation of ephedrine significantly. This is likely a oubain effect only since a similar decrease in uptake occurred when 10^{-4} oubain was present in normal Krebs solution.

The effect of low temperature (4°C) is shown in table 7. It can be seen that the accumulation of ephedrine in the tissue is reduced to approximately the same level as in the incubation media. The effect of low temperature (4°C) on the accumulation of ephedrine is much greater than that produced by oubain or low Na^+ or by any other drug used so far.

(J) *Effect of Temperature on Ephedrine Accumulation*

Active transport may be indicated if the transport system possesses a high temperature coefficient (Q_{10}). Figure 12 shows the effect of temperature on ephedrine accumulation under three different conditions; in Krebs solution only, in Krebs solution containing 10^{-2} M phentermine, and in low Na^+ Krebs containing 2×10^{-5} M cocaine. Although the usual effect of temperature was noted in all cases, that is, an enhanced uptake with increased temperature, the effect was not great. The calculated Q_{10} values for all conditions varied from 1.1 to 1.2 and is consistent with a passive diffusion mechanism. Alternately one can assume that the size of the "insensitive component" could be indicated by the amount of ephedrine accumulated in the tissue in the presence of 10^{-2} M phentermine, the drug which caused the greatest inhibition. On this basis the calculated Q_{10} value is 1.9 which indicates the possible

TABLE 7

EFFECT OF K^+-FREE MEDIA AND LOW TEMPERATURE

Tissues were incubated for 30 min in the treatments shown 5×10^{-7} M ephedrine was added for another 30 min. Mean ± S.E. of 8 observations.

TREATMENT	T/M	t-TEST
Control (Normal Krebs, 37°C)	2.13 ± 0.06	
K^+-free Medium	2.06 ± 0.04	NS
K^+-free medium + 10^{-4} M oubain	1.91 ± 0.06	$p < 0.02$
Oubain, 10^{-4} M (Normal Krebs)	1.91 ± 0.05	$p < 0.02$
4°C (ice)	0.96 ± 0.04	$p < 0.001$

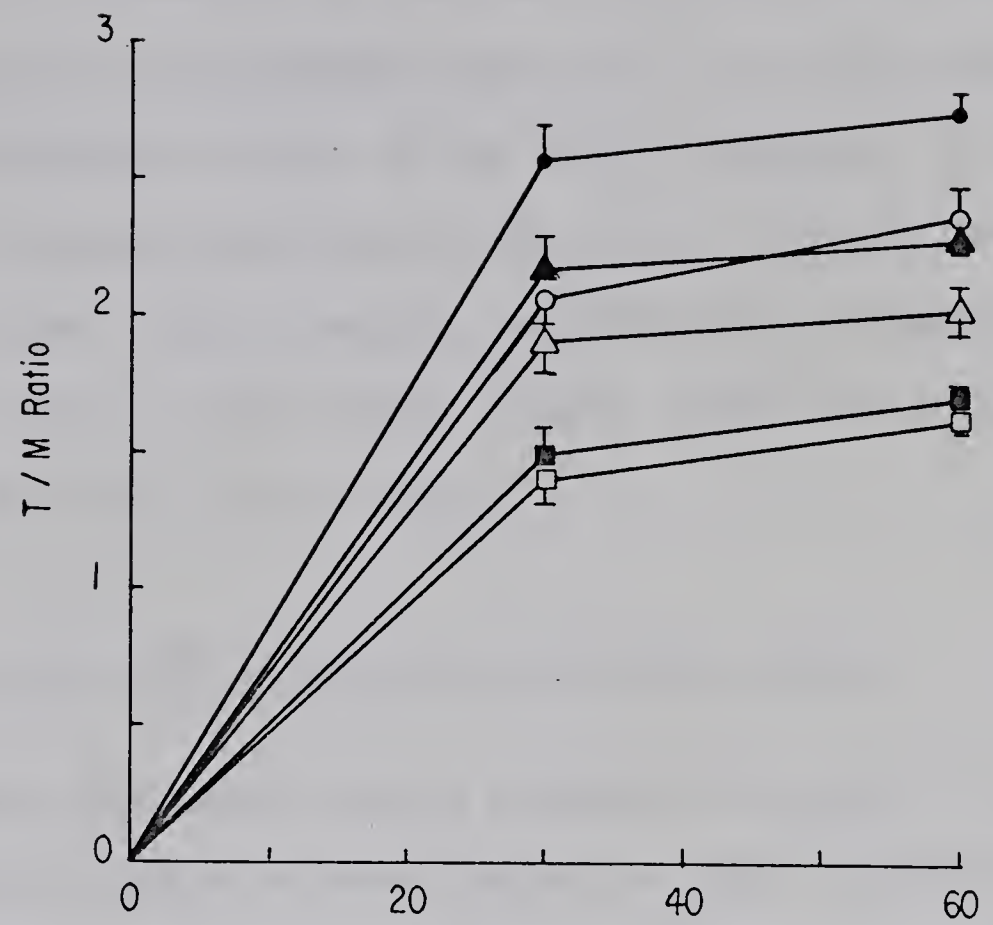


FIGURE 12. Effect of temperature on ephedrine accumulation. Tissues were preincubated in Krebs solution under the following conditions: ●——●, normal Krebs at 37°C; o——o, normal Krebs at 27°C; ■——■, phentermine (10^{-2} M) at 37°C; □——□, phentermine (10^{-2} M) at 27°C; ▲——▲, low Na^+ + cocaine (2×10^{-5} M) at 37°C; Δ——Δ, low Na^+ + cocaine (2×10^{-5} M) at 27°C. 5×10^{-7} M ^{14}C-ephedrine was added for an additional 30 min. Drugs and conditions present throughout. Mean values ± S.E. of 6 observations.

existence of an active transport system for ephedrine uptake. The problem with this assumption is that there is no evidence that more than one component exists for ephedrine transport. No useful information is obtained in estimating the size of the "active component" by calculating the amount of ephedrine inhibited in the presence of both low Na^+ and 2×10^{-5} M cocaine. This is because the effect of a 10 degree drop in temperature to 27°C in normal Krebs solution is the same or greater than that produced by low Na^+ plus cocaine.

(K) Effect of Metabolic Inhibitors on Ephedrine Uptake

Energy from aerobic and/or anaerobic glycolysis is required in many transport systems to drive the Sodium Pump. Table 8 shows the effect of metabolic inhibitors on the uptake (expressed as the T/M ratio) of ephedrine. It is seen that ephedrine uptake is not affected by the presence of metabolic inhibitors either by themselves or in combination to inhibit both aerobic and anaerobic pathways. Although the accumulation of ephedrine appeared to be significantly reduced when both glycolysis and oxidation were inhibited by dinitrophenol and iodoacetate, this decrease can be accounted by the swelling of the tissues (see table 1). These results indicate that energy is not required for ephedrine transport.

(L) Effect of 6-Hydroxydopamine Pretreatment on Ephedrine Accumulation

6-Hydroxydopamine produces an efficient and extremely long-lasting depletion of noradrenaline due to the selective destruction of sympathetic nerve endings (Tranzer and Thoenen, 1967). To show whether

TABLE 8

EFFECT OF METABOLIC INHIBITORS ON EPHEDRINE ACCUMULATION

Tissues were preincubated in the treatments shown for 30 min and then 5 x 10^{-7} M ^{14}C-ephedrine was added for 30 additional minutes. Iodoacetate was present in the preincubation period only. Mean ± S.E. of 6 observations. Figures in brackets indicate molar concentration of metabolic inhibitors.

TREATMENT	T/M	t-TEST
Control	2.02±0.11	
2-deoxy-D-glucose (2 x 10^{-3} M)	2.13±0.14	NS
Iodoacetate (10^{-3} M)	1.96±0.11	NS
Glucose-free	1.90±0.10	NS
Glucose-free + 2-deoxy-D-glucose(10mM)	2.08±0.07	NS
Glucose-free + Iodoacetate (10^{-3} M)	1.90±0.08	NS
2,4-Dinitrophenol (5 x 10^{-4} M)	1.98±0.17	NS
Sodium Azide (10^{-3} M)	1.99±0.09	NS
2,4-Dinitrophenol (5 x 10^{-4} M) + Iodoacetate (10^{-3} M)	1.70±0.06	$p<0.005$
Glucose-free + 2,4-Dinitrophenol (5x10^{-4}M)	1.86±0.09	NS

the accumulation of ephedrine can be linked to functionally intact nerve endings the effect of pretreating the rabbits with 6-hydroxydopamine was studied. It can be seen from the results shown in table 9 that denervation had no effect on ephedrine uptake. Accumulation of ephedrine by the tissues of pretreated rabbits was similarly unaffected in the presence of other drugs.

In order to show that the rabbits were adequately denervated, the uptake of metaraminol, an amine known to be transported into sympathetic nerve terminals, was studied. The results in table 10 show that the rabbits were denervated since the uptake of metaraminol was considerably reduced in the tissues of rabbits pretreated with 6-hydroxydopamine.

TABLE 9

EFFECT OF DENERVATION ON EPHEDRINE ACCUMULATION

Tissues were preincubated 30 min under the treatments shown. 5×10^{-7} M ephedrine was added for an additional 30 min. Mean ± S.E. of 6 observations. Figures in brackets indicate molar concentration of the drugs.

TREATMENT	UNTREATED RABBIT	DENERVATED RABBIT	t-TEST
Normal Krebs	2.06 ± 0.04	2.03 ± 0.05	NS
Cocaine (2×10^{-5} M)	1.95 ± 0.12	1.82 ± 0.06	NS
Oubain (10^{-4} M)	1.94 ± 0.06	1.89 ± 0.10	NS
Phentermine (10^{-3})	1.59 ± 0.04	1.51 ± 0.06	NS
Phenylpropanolamine(10^{-3})	1.66 ± 0.03	1.57 ± 0.07	NS
Phenylethanolamine (10^{-3})	1.76 ± 0.05	1.64 ± 0.06	NS
Phenoxybenzamine (10^{-5})	2.00 ± 0.05	1.95 ± 0.10	NS
Low Na^+ Krebs	1.79 ± 0.10	1.67 ± 0.05	NS

TABLE 10

EFFECT OF DENERVATION ON METARAMINOL ACCUMULATION

Tissues were incubated for 30 min at 37°C in Krebs solution containing 2.5×10^{-7} M ^{3}H-metaraminol. Mean values ± S.E. of 6 observations.

TISSUE	T/M	t-TEST
Untreated rabbit (Control)	4.86 ± 0.44	
Denervated rabbit	1.42 ± 0.08	$p < 0.001$

V. DISCUSSION

The uptake experiments indicate that the accumulation of ephedrine is concentration- and time-dependent. Slices of rabbit atria are able to take up and accumulate ephedrine from the incubation medium against a concentration gradient. A rapid accumulation of ephedrine occurs since in the uptake experiments, the amount of ephedrine in the tissue exceeds that in the medium by 7 min and a steady state level is reached within one hour. This study in which rabbit atria slices were used and the study by Jacquot *et al.* (1969) in which the isolated rat heart was used showed that ephedrine was accumulated to levels of 2.7 and 3.0 times those of the medium containing a low concentration of the amine. These concentration ratios proved lower than those found for noradrenaline. For instance, noradrenaline was accumulated to levels up to 5 times those in the medium containing low concentrations of noradrenaline by rat brain or heart slices (Dengler *et al.*, 1961b). More impressive concentration ratios, however, were obtained in perfused tissues. Iversen (1963) showed that the isolated rat heart, perfused with a medium containing low concentrations of noradrenaline (10 ng/ml), accumulated the amine to levels greater than 10 times that in the perfusing medium.

Although ephedrine is rapidly accumulated by rabbit atria slices, the concentration ratio (T/M ratio) of the amine in the tissue to that in the incubation medium was only slightly diminished by a 10-fold or greater increase in the concentration of amine in the incubation medium. This finding is similar to that found for amphetamine,

an amine which like ephedrine lacks phenolic hydroxyl groups (Ross and Renyi, 1966b) but different to that found for noradrenaline where the T/M ratio was considerably reduced (Dengler *et al.*, 1961b; Ross and Renyi, 1964).

Iversen (1963, 1965b) described the existence of two uptake processes for noradrenaline. The first process, $Uptake_1$, was considered to operate at low concentrations of noradrenaline in the medium whereas the second process, $Uptake_2$, operates at high amine concentrations. Both processes are saturable with increasing external amine concentrations and are described by the classical Michealis-Menten equation for enzyme kinetics. The transport of ephedrine by rabbit atria slices is clearly different in this respect. For instance there appears to be evidence for no more than one uptake process for ephedrine in this study and the accumulation of the amine occurred at an approximately uniform rate even at the highest concentration used and could not be described by the Michealis-Menten equation and thus indicating non-saturation or passive diffusion.

This study showed that a wide variety of amines inhibited ephedrine accumulation. A number of interesting structure activity relations emerged. First of all, phentermine, an amine lacking in phenolic hydroxyl groups caused a greater inhibition of ephedrine transport than did the amines known for their potent effects on the well known noradrenaline transport systems, $Uptake_1$ and $Uptake_2$. Metaraminol, a very powerful $Uptake_1$ inhibitor; noradrenaline, also a potent $Uptake_1$ inhibitor and at the concentration used would also be a good $Uptake_2$ inhibitor; and isoproterenol, a potent $Uptake_2$ inhibitor

all caused a significant inhibition of ephedrine accumulation. That transport of ephedrine occurs by $Uptake_2$ is unlikely since normetanephrine, a potent $Uptake_2$ inhibitor, was not effective. The inhibitory effect of isoproterenol may be due to its small ability to inhibit $Uptake_1$ which at the concentration of the amine used may become significant. Alternately the effect of isoproterenol and the other amines may be non-specific. This finding differs from that of Jacquot *et al.* (1969) who found that noradrenaline was without effect on ephedrine accumulation. However, they used lower concentrations of noradrenaline equal to that of labelled ephedrine ($\simeq 5 \times 10^{-7}$ M) and thus may account for the lack of effect.

A second feature is that extremely high concentrations of all amines had to be used to inhibit ephedrine accumulation significantly (table 2). Although phentermine of the group of amines studied in table 2 appeared to inhibit the accumulation of ephedrine more markedly than the other amines, its effect was not striking. Subsequently (figure 8) it was shown that a 20-fold increase in the concentration of phentermine (10^{-5} M) in the incubation medium over that of ^{14}C-ephedrine (5×10^{-7} M) did not even cause any apparent inhibition of ephedrine transport. The study of Burgen and Iversen (1965) in contrast, showed that many of the amines inhibited noradrenaline transport markedly when they were present in the medium in 10-fold or less the molar concentrations than was noradrenaline.

Another feature is that the absence of phenolic hydroxyl groups enhanced the ability of amines to act as inhibitors of ephedrine transport. The effect of adding another phenolic hydroxyl group to phenol-

ethylamines, however, did not have any additional inhibitory effect since amines with one or two hydroxyl groups inhibited ephedrine uptake with a smaller but equal potency. It is interesting to note that phenol- and catecholpropanolamines related to amphetamine, *e.g.*, para-hydroxyamphetamine and α-methyldopamine, appear to be more potent inhibitors of ephedrine uptake relative to the other amines possessing phenolic hydroxyl groups, *e.g.*, tyramine and dopamine in particular. One possibility that may account for this difference is that tyramine and dopamine are metabolized by the enzyme, monoamine oxidase, and thus may not appear to be as effective whereas parahydroxyamphetamine and α-methyldopamine are protected from metabolism by monoamine oxidase by the α-methyl group. Alternatively this effect could be due to the presence of the α-methyl group *per se*. However, this is unlikely since another group of α-methylated amines, metaraminol and α-methylnoradrenaline, inhibit the uptake of ephedrine very little or not at all. The structural specificity for ephedrine appears to be different from that found for noradrenaline by Burgen and Iversen (1965) in the isolated rat heart. They found that the presence of hydroxyl groups enhanced the affinity of the amine for the $Uptake_1$ site. It is difficult to compare the results obtained in this study with those obtained by Burgen and Iversen, however, because a different species of animal was used, a much higher concentration of the amines had to be used in order to significantly inhibit ephedrine transport and the small amount that ephedrine accumulation was inhibited prevented the use of the probit plot method.

The results obtained with (±)-, (-)-, (-)-ψ-, and (+)-ψ-ephedrine and (+)- and (-)-amphetamine isomers show that the uptake system for

ephedrine does not show any stereochemical specificity. This is unlike the noradrenaline $Uptake_1$ system in rat heart which was found by Iversen (1963) and Burgen and Iversen (1965) to exhibit stereochemical specificity. The isomers (-)-noradrenaline and (+)-amphetamine were found to be more effective $Uptake_1$ inhibitors than (+)-noradrenaline and (-)-amphetamine. he $Uptake_2$ system for noradrenaline did not, however, discriminate between the optical isomers (Iversen, 1965).

Cocaine and desipramine, both selective $Uptake_1$ inhibitors significantly inhibited ephedrine accumulation by rabbit atria slices at lower concentrations than lignocaine. The possibility that the inhibition of ephedrine transport is due to a local anesthetic effect is thus unlikely. Although the results could indicate an $Uptake_1$ process, other alternatives must be considered. It is possible that the transport of ephedrine occurs by a passive diffusion process followed by binding and that the lipophilic properties of cocaine and desipramine interfere with the binding of ephedrine. Another alternative is that these drugs interfere with the movement of ephedrine across cell membranes. There is yet, however, no evidence that these drugs bind to tissue or interfere with movements of other substances across cell membranes. The third alternative is that these drugs inhibit ephedrine transport by a non-specific mechanism. This alternative can account for the small and gradual inhibitory effects observed with these drugs and lignocaine and also account for the observation that desipramine is not more potent than cocaine. Jacquot *et al.* (1969) found that cocaine at a concentration of 3×10^{-5} M did not affect the accumulation of ephedrine by the isolated rat heart. The difference in findings may be due to the

different techniques used and also because a different species of animal was used.

The transport of ephedrine notably differs from that of noradrenaline in that the adrenergic receptor blocking drugs did not inhibit the uptake of ephedrine. Phenoxybenzamine at the concentration used would have blocked both $Uptake_1$ and $Uptake_2$ whereas SKF-550 would have blocked only $Uptake_2$. The two steroids, estradiol and testosterone, both potent $Uptake_2$ inhibitors, inhibited ephedrine accumulation significantly. It is more likely that this inhibition like that of cocaine is non-specific. There is also no evidence that steroids bind to various tissues and interfere with the passage of other substances across cell membranes. The possibility that the transport of ephedrine occurs by neither $Uptake_1$ nor $Uptake_2$ must be considered. One other possibility, that ephedrine can accumulate in collagen tissue is unlikely since oxytetracycline failed to inhibit ephedrine transport.

In the present study the inhibitory action of oubain on ephedrine transport was slight and significant only at high oubain concentrations of 10^{-4} - 10^{-3} M. Sodium was found to enhance the uptake of ephedrine but was not an absolute requirement and secondly a large fraction (over 80%) of ephedrine still accumulated in the presence of low Na^+. Furthermore no other ions were able to substitute for sodium. This indicated that there may be two mechanisms involved in the transport of ephedrine. From these data, it can be postulated that a small fraction of ephedrine is transported actively by a co-transport mechanism with sodium. Supporting evidence for such an active transport mechanism is that the uptake of the amine was reduced in the tissue to the same

levels as in the medium at a temperature of 4°C. The uphill transport of ephedrine by the tissue is further supporting evidence for active transport. However, the results obtained in the initial rates experiment showed only one large component which is possibly passive. One way in which the two mechanisms may co-exist is that the co-transport mechanism, being very small, would be masked by the other mechanism which also would have a high affinity for ephedrine. The estimated Q_{10} value for ephedrine transport assuming an insensitive component which is the one not inhibited by phentermine is high enough to indicate the involvement of such a co-transport system. The problem is that there is no evidence for the existence of more than one component for ephedrine transport.

Thoenen *et al.* (1968) similarly found in the isolated perfused rat heart that the accumulation of amphetamine which like ephedrine also lacks phenolic hydroxyl groups and thus may be expected to be transported in a like manner was sodium and temperature dependent. They proposed that this difference is due to impaired tissue perfusion resulting from vascular constriction brought about by reduced sodium and temperature. This reason cannot account for the sodium and temperature dependence of ephedrine transport in our studies since slices of rabbit atria were used.

Energy is needed to drive the Sodium Pump and is supplied by anaerobic and/or aerobic glycolysis. The failure to decrease ephedrine uptake by the metabolic inhibitors, dinitrophenol, iodoacetate, or azide, either by themselves or in a combination which would inhibit both the glycolytic and oxidative pathways, indicated that energy is not required for ephedrine transport. The findings that metabolic energy is not

required to transport ephedrine and that potassium-free medium is not able to prevent the uptake of ephedrine make it unlikely that ephedrine transport occurs by an active transport mechanism.

Selective destruction of sympathetic nerve endings (or $Uptake_1$) by chemical denervation with 6-hydroxydopamine failed to decrease ephedrine accumulation. The lack of dependence of functionally intact sympathetic nerves on ephedrine accumulation is the most important difference between the transport systems of noradrenaline and ephedrine. This means, first of all, that the bulk of the ephedrine accumulated in the rabbit atria must be located extraneuronally. These results do not indicate that sympathetic nerves are incapable of accumulating ephedrine or that sympathetic nerves are capable of concentrating the amine to a lesser or greater extent than extraneuronal tissue. This is because the sympathetic nerve terminals occupy an extremely small volume of the total tissue and therefore accumulation of any substance has to be several magnitudes higher than that in the medium before it can be evident in the T/M ratio. Noradrenaline has been shown to accumulate almost exclusively in adrenergic nerve terminals (Iversen, 1963). Therefore an accumulation of ephedrine by adrenergic nerve terminals of the same order of magnitude as that found for noradrenaline can be excluded.

The question that remains at this point is: How is ephedrine transported? The following points are briefly reviewed. Transport of ephedrine by $Uptake_2$ is unlikely because normetanephrine, phenoxybenzamine and SKF-550 did not inhibit ephedrine accumulation. Binding of ephedrine to collagen is unlikely because oxytetracycline did not inhibit ephedrine accumulation. Even though the process is an uphill one,

oubain-sensitive to a small degree and sodium and temperature dependent, active transport of ephedrine with and without co-transport with sodium is unlikely since it was non-saturable, did not require metabolic energy and was not affected by potassium-free medium. $Uptake_1$ is unlikely since chemical denervation by 6-hydroxydopamine failed to inhibit ephedrine accumulation.

The possibility that ephedrine transport occurs by passive diffusion followed by binding must be considered. The view that ephedrine is accumulated and then bound to non-specific sites is strongly supported by the differences found between its transport and that of noradrenaline. Transport of ephedrine is clearly different from that of noradrenaline. The accumulation of ephedrine was non-saturable, did not exhibit stereochemical specificity, showed only one component, needed high concentrations of amines to inhibit uptake, T/M ratios were only slightly diminished with 10-fold or even greater increases in amine concentration, unaffected by most $Uptake_2$ inhibitors and not impaired by a combined inhibition of aerobic and anaerobic glycolysis. The high concentrations of amines, cocaine, desipramine and oubain required to achieve an inhibitory effect on ephedrine accumulation is further supporting for a passive process.

The finding, however, that ephedrine did not accumulate significantly in adrenergic nerve terminals does not permit the definite conclusion that the amine cannot be transported by the noradrenaline uptake system. A significant uptake of ephedrine still could be masked by its rapid outward diffusion from the neurone because of its high lipophilic properties as suggested by Thoenen *et al.* (1968) or be

masked by a large extraneuronal uptake as suggested by Trendelenburg (1972) to account for the indirect actions of these amines. The suggestion by Paton (1974) that the highly lipophilic properties of the amines enable them to diffuse passively across adrenergic nerve terminals, replacing noradrenaline from the storage vesicles which effluxes from the neurone by a cocaine-sensitive carrier-mediated process is a possibility in keeping with our findings in this study.

VI. BIBLIOGRAPHY

AMATSU, H. AND KUBOTA, S. (1918). Pharmacological action of ephedrine and mydriatin. Chem. Abst. 12: 2019.

ANDÉN, N.E. (1964). Uptake and release of dextro- and levo-adrenaline in noradrenaline stores. Acta Pharmacol. Tox. 21: 59-75.

AXELROD, J. (1953). Studies on sympathomimetic amines. I. The biotransformation and physiological disposition of l-ephedrine and l-norephedrine. J. Pharmacol. exp. Ther. 109: 62-73.

AXELROD, J., HERTTING, G. AND POTTER, L. (1962). Effect of drugs on the uptake and release of ^{3}H-norepinephrine in the rat heart. Nature (Lond.). 194: 297.

AXELROD, J. AND TOMCHICK, R. (1960). Increased rate of metabolism of epinephrine and norepinephrine by sympathomimetic amines. J. Pharmacol. exp. Ther. 130: 367-369.

AXELROD, J., WEIL-MALHERBE, H. AND TOMCHICK, R. (1959). The physiological disposition of ^{3}H-epinephrine and its metabolite metanephrine. J. Pharmacol. exp. Ther. 127: 251-256.

AXELROD, J., WHITBY, L.G. AND HERTTING, G. (1961). Effect of psychotropic drugs on the uptake of ^{3}H-norepinephrine by tissues. Science. 133: 383-384.

BARGER, G. AND DALE, H.H. (1910). Chemical structure and sympathomimetic action of amines. J. Physiol. 41: 19-59.

BERNDT, W.O. AND BEECHWOOD, E.C. (1965). Influence of inorganic electrolytes and oubain on uric acid transport. Am. J. Physiol. 208: 642-648.

BERTI, F. AND SHORE, P.A. (1967). A kinetic analysis of drugs that inhibit the adrenergic neuronal membrane amine pump. Biochem. Pharmacol. 16: 2091-2094.

BOGDANSKI, D.F. AND BRODIE, B.B. (1966). Role of sodium and potassium ions in storage of norepinephrine by sympathetic nerve endings. Life Sci. 5: 1563-1569.

BOGDANSKI, D.F. AND BRODIE, B.B. (1969). The effects of inorganic ions on the storage and uptake of ^{3}H-norepinephrine by rat heart slices. J. Pharmacol. exp. Ther. 165: 181-189.

BOGDANSKI, D.F., TISSARI, A. AND BRODIE, B.B. (1968). Role of sodium, potassium, oubain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. Life Sci. 7: 419-428.

BRANSOME, E.D. (1970). The current status of liquid scintillation counting. Grune and Stratton. New York.

BURGEN, A.S.V. AND IVERSEN, L.L. (1965). The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. Brit. J. Pharmacol. Chemother. 25: 34-49.

BURN, J.H. (1932). The action of tyramine and ephedrine. J. Pharmacol. exp. Ther. 46: 75-95.

BURN, J.H. AND TAINTER, M.L. (1931). An analysis of the effect of cocaine on the action of adrenaline and tyramine. J.Physiol. 71: 169-193.

CARLSSON, A., HILLARP, N-Å. AND WALDECK, B. (1963). Analysis of the Mg^{++}-ATP dependent storage mechanisms in the amine granules of the adrenal medulla. Acta Physiol. Scand. 215: (Suppl.) 59.

CARLSSON, A. AND WALDECK, B. (1965). Inhibition of ^{3}H-metaraminol uptake by antidepressive and related agents. J. Pharm. Pharmacol. 17: 243-244.

CHEN, K.K. AND SCHMIDT, C.F. (1925). The action of ephedrine, the active principle of the Chinese drug Ma Huang. J. Pharmacol. exp. Ther. 24: 339-357.

CHEN, K.K. AND SCHMIDT, C.F. (1930). Ephedrine and Related Substances. The Williams and Wilkins Co. (Baltimore).

COLBURN, R.W., GOODWIN, F.K., MURPHY, D.L., BUNNEY, W.E. AND DAVIS, J.M. (1968). Quantitative studies of norepinephrine uptake by synaptosomes. Biochem. Pharmacol. 17: 957-964.

CRANE, R.K. (1965). Na^+-dependent transport in the intestine and other animal tissue. Fed. Proc. 24: 1000-1006.

CROUT, J.R. (1964). The uptake and release of ^3H-norepinephrine by the guinea pig heart *in vivo*. Arch. exp. Path. Pharmak. 248: 85-98.

CSAKY, T.Z. AND HARA, Y. (1965). Inhibition of active intestinal sugar transport by digitalis. Am. J. Physiol. 209: 467-472.

DENGLER, H.J., MICHEALSON, I.A., SPIEGEL, H.E., AND TITUS, E. (1962). The uptake of labelled norepinephrine by isolated brain and other tissues of the cat. Int. J. Neuropharmac. 1: 23-38.

DENGLER, H.J., SPIEGEL, H.E. AND TITUS, E.O. (1961a). Effects of drugs on uptake of isotopic norepinephrine. Nature (Lond.). 191: 816-817.

DENGLER, H.J., SPIEGEL, H.E. AND TITUS, E.O. (1961b). Uptake of tritium-labelled norepinephrine in brain and other tissues of the cat *in vitro*. Science. 133: 1072-1073.

DRASKOCZY, P.R. AND TRENDELENBURG, U. (1968). The uptake of l- and d-norepinephinre by the isolated perfused rabbit heart in relation to the stereospecificity of the sensitizing action of cocaine. J. Pharmacol. exp. Ther. 159: 66-73.

FISCHER, J.E., KOPIN, I.J. AND AXELROD, J. (1965). Evidence for extra-neuronal binding of norepinephrine. J. Pharmacol. exp. Ther. 147: 181-185.

FLECKENSTEIN, A. AND BASS, H. (1953). Zum Mechanismus der Wirkungsverstärkung und Wirkungsabschwächung der Katzen-Nickhaut für Sympathomimetica der Brenzkatechin-Reihe. Arch. exp. Path. Pharmak. 220: 143-156.

FLECKENSTEIN, A. AND BURN, J.H. (1953). The effect of denervation on the action of sympathomimetic amines on the nictitating membrane. Brit. J. Pharmacol. 8: 69-78.

FLECKENSTEIN, A. AND STOCKLE, D. (1955). Zum mechanismus der Wirkungsverstärkung und Wirkungsabschwächung sympathomimetischer Amine durch Cocain und andere Pharmacka. II. Die Hemmung der Neurosympathomimetica durch Cocain. Arch. exp. Path. Pharmak. 224: 401-415.

FLEMING, R.M. AND CLARK, W.G. (1970). Quantitative thin-layer chromatographic estimation of labelled dopamine and norepinephrine, their precursors and metabolites. J. Chromatog. 52: 305-312.

FRÖHLICH, A. AND LOEWI, O. (1910). Untersuchungen zur Physiologie und Pharmakologie des vegetativen Nervensystems. II. Mitteilung: Über eine Steigerung der Adrenalineimpfindlich Keit durch Cocain. Arch. exp. Path. Pharmak. 62: 159-169.

FURCHGOTT, R.F. (1955). The pharmacology of vascular smooth muscle. Pharmacol. Rev. 7: 183-265.

GIACHETTI, A. AND SHORE, P.A. (1966). Studies *in vitro* of amine-uptake mechanisms in heart. Biochem. Pharmacol. 15: 607-614.

GILLIS, C.N. AND PATON, D.M. (1967). Cation dependence of sympathetic transmitter retention by slices of rat ventricle. Brit. J. Pharmacol. Chemother. 29: 309-318.

GLYNN, I.M. (1964). The action of cardiac glycosides on ion movements. Pharmacol. Rev. 16: 381-407.

GREEN, R.D. AND MILLER, J.W. (1966). Evidence for the active transport of epinephrine and norepinephrine by the uterus of the rat. J. Pharmacol. exp. Ther. 152: 42-50.

HAMBERGER, B. (1967). Reserpine resistant uptake of catecholamines in isolated tissues of the rat. Acta Physiol. Scand. Suppl. 295.

HARDMAN, J.G. AND MAYER, S.E. (1965). The influence of cocaine on some metabolic effects and the distribution of catecholamines. J. Pharmacol. exp. Ther. 148: 29-39.

HERTTING, G. AND AXELROD, J. (1961). Fate of tritiated noradrenaline at the sympathetic nerve endings. Nature (Lond.). 192: 172-173.

HERTTING, G., AXELROD, J., KOPIN, I.J. AND WHITBY, L.G. (1961a). Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. Nature (Lond.). 189: 66.

HERTTING, G., AXELROD, J. AND WHITBY, L.G. (1961b). Effects of drugs on the uptake and metabolism of ^{3}H-norepinephrine. J. Pharmacol. exp. Ther. 134: 146-153.

HERTTING, G., AXELROD, J. AND PATRICK, R.W. (1962). Actions of bretylium and guanethidine on the uptake and release of ^{3}H-norepinephrine. Brit. J. Pharmacol. 18: 161-166.

HILLARP, N.Å. AND MALMFORS, T. (1964). Reserpine and cocaine blocking of the uptake and storage mechanisms in adrenergic nerves. Life Sciences. 3: 703-708.

HIROSE, M. (1915). Ueber die pharmakologischen Eigenschaften einigen dem Adrenalin nahestehender Substanzen, o-dioxyphenylaethanolamin, Phenylaethanolamin, Ephedrin und Mydriatin. Meit. Med. Fakul. Kaiser Univ. Tokyo. 13: 479-506.

IGNARRO, L.J. AND SHIDEMAN, F.E. (1968). The requirement of sympathetic innervation for the active transport of norepinephrine by the heart. J. Pharmacol. exp. Ther. 159: 59-65.

IVERSEN, L.L. (1963). The uptake of noradrenaline by the perfused rat heart. Brit. J. Pharmacol. Chemother. 21: 523-537.

IVERSEN, L.L. (1964). The inhibition of noradrenaline uptake by sympathomimetic amines. J. Pharm. Pharmacol. 16: 435-437.

IVERSEN, L.L. (1965a). The uptake of adrenaline by the rat isolated heart. Brit. J. Pharmacol. 24: 387-394.

IVERSEN, L.L. (1965b). The uptake of catecholamines at high perfusion concentrations in rat isolated heart: a novel catecholamine uptake process. Brit. J. Pharmacol. Chemother. 25: 18-23.

IVERSEN, L.L. (1965c). Inhibition of noradrenaline uptake by drugs. J. Pharm. Pharmacol. 17: 62-64.

IVERSEN, L.L. (1966). Accumulation of α-methyltyramine by the noradrenaline uptake process in the isolated rat heart. J. Pharm. Pharmacol. 18: 481-484.

IVERSEN, L.L. (1967). The uptake and storage of noradrenaline in sympathetic nerves. London: Cambridge University Press.

IVERSEN, L.L. AND KRAVITZ, E.A. (1966). Sodium dependence of transmitter uptake of adrenergic nerve terminals. Molec. Pharmacol. 2: 360-362.

IVERSEN, L.L. AND SALT, P.J. (1970). Inhibition of catecholamine Uptake$_2$ by steroids in the isolated rat heart. Brit. J. Pharmacol. 40: 528-530.

IVERSEN, L.L., SALT, P.J. AND WILSON, H.A. (1972). Inhibition of catecholamine uptake in the isolated rat heart by haloalkylamines related to phenoxybenzamine. Brit. J. Pharmacol. 46: 647-657.

JACQUOT, C., BRALET, J., COHEN, Y. AND VALETTE, G. (1969). Fixation de la dl-ephedrine-^{14}C par le coeur isolé perfusé de rat. Biochem. Pharmacol. 18: 903-914.

KIPNIS, D.M. AND PARRISH, J.E. (1965). Role of Na^+ and K^+ on sugar (2-deoxy glucose) and amino acid (α-aminoisobutyric acid) transport in striated muscle. Fed. Proc. 24: 1051-1059.

KIRPEKAR, S.M. AND WAKADE, A.R. (1968). Factors influencing noradrenaline uptake by perfused spleen of the cat. J. Physiol. (Lond.). 194: 609-626.

KOPIN, I.J. AND BRIDGERS, W. (1963). Differences in D- and L-norepinephrine-^{3}H. Life Sci. 2: 356-362.

LEFFLER, E.B., SPENCER, I.M. AND BURGER, A. (1951). Dissociation constants of adrenergic amines. J. Am. Chem. Soc. 73: 2611-2613.

LIGHTMAN, S.L. AND IVERSEN, L.L. (1969). The role of Uptake$_2$ in the extraneuronal metabolism of catecholamines in the isolated rat heart. Brit. J. Pharmacol. 37: 638-649.

LINDMAR, R. AND MUSCHOLL, E. (1964). Die Wirkung von Pharmaka auf die Elimination von Noradrenalin aus der Perfusionsflüssigkeit und die Noradrenalin Aufnahme in das isolierte Herz. Arch. exp. Path. Pharmak. 247: 469-492.

LINDMAR, R. AND MUSCHOLL, E. (1965). Die Aufnahme von α-methylnoradrenalin in das isolierte Kaninchenherz und seine freisetzung durch Reserpin und Guanethidin *in vivo*. Arch. exp. Path. Pharmak. 249: 529-548.

MACMILLAN, W.H. (1959). A hypothesis concerning the effect of cocaine on the action of sympathomimetic amines. Brit. J. Pharmacol. 14: 385-391.

MAICKEL, R.P., BEAVEN, M.A. AND BRODIE, B.B. (1963). Implications of uptake and storage of norepinephrine by sympathetic nerve endings. Life Sci. 2: 953-958.

MARLEY, E. (1962). Action of some sympathomimetic amines on the cat's iris, *in situ* or isolated. J. Physiol. (Lond.). 162: 193-211.

MAXWELL, R.A., PLUMMER, A.J., PAVALSKI, H. AND SCHNEIDER, F. (1960). Concerning a possible action of guanethidine (54-5864) in smooth muscle. J. Pharmacol. exp. Ther. 129: 24-30.

MAXWELL, R.A., POVALSKI, H. AND PLUMMER, A.J. (1959). A differential effect of reserpine on the pressor amine activity and its relationship to other agents producing this effect. J. Pharmacol. exp. Ther. 125: 178-183.

MIURA, K. (1887). Vorläufige Mitteilung über Ephedrin, ein neues Mydriaticum. Berl. Klin Wochschr. 24: 707.

MUSCHOLL, E. (1960). Die Hemmung der Noradrenalin-Aufnahme des Herzens durch Reserpin und die Wirkung von Tyramin. Arch. exp. Path. Pharmak. 240: 234-241.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. Brit. J. Pharmacol. Chemother. 16: 352-359.

MUSCHOLL, E. AND WEBER, E. (1965). Die Hemmung der Aufnahme von α-methyl-noradrenalin in das Herz durch sympathomimetische Amine. Arch. exp. Path. Pharmak. 252: 134-143.

NAGAI, N. (1887). Ephedrin. Pharm. Zeit. 32: 700.

PARRISH, J.E. AND KIPNIS, D.M. (1964). Effect of Na^+ on sugar and amino acid transport in striated muscle. J. Clin. Invest. 43: 1994-2002.

PATIL, P.N., TYE, A. AND LAPIDUS, J.B. (1965). A pharmacological study of the ephedrine isomers. J. Pharmacol. exp. Ther. 148: 158-168.

PATON, D.M. (1968). The cation and metabolic dependence of metaraminol retention by rat uterine horns. Brit. J. Pharmacol. Chemother. 33: 277-286.

PATON, D.M. (1971). Effects of Na^+ and K^+ on the uptake of metaraminol by rabbit ventricular slices. Brit. J. Pharmacol. 41: 65-75.

PATON, D.M. (1972). Metabolic requirements for the uptake of noradrenaline by isolated atria and vas deferens of the rabbit. Pharmac. 7: 78-88

PATON, D.M. (1974). Mechanism of inhibition by cocaine of action of indirectly acting sympathomimetic amines. Am. Heart J. 88: 128-129.

PLETSCHER, A., BURKARD, W.P., TANZER, J.P., AND GEY, K.F. (1967). Two sites of 5-hydroxytryptamine uptake in blood platelets. Life Sci. 6: 273-280.

POVALSKI, H.J. AND GOLDSMITH, E.D. (1959). Effect of methylphenidate on cardiovascular actions of pressor amines. Proc. Soc. exp. Biol. and Med. 101: 717-721.

POWIS, G. (1973). Binding of catecholamines to connective tissue and the effect upon the responses of blood vessels to noradrenaline and to nerve stimulation. J. Physiol. (Lond.). 234: 145-162.

RAAB, W. AND GIGEE, W. (1955). Specific avidity of heart muscle to absorb and store epinephrine and norepinephrine. Circ. Res. 3: 553-558.

ROSS, S.B. AND RENYI, A.L. (1964). Blocking action of sympathomimetic amines on the uptake of tritiated noradrenaline by mouse cerebral cortex tissues. Acta Pharmacol. Tox. 21: 226-239.

ROSS, S.B. AND RENYI, A.L. (1966a). Uptake of tritiated tyramine and (+)-amphetamine by mouse heart slices. J. Pharm. Pharmacol. 18: 756-757.

ROSS, S.B. AND RENYI, A.L. (1966b). Uptake of some tritiated sympathomimetic amines by mouse brain cortex slices *in vitro*. Acta Pharmacol. Tox. 24: 297-309.

ROSS, S.B. AND RENYI, A.L. (1971). Uptake and metabolism of β-phenethylamine and tyramine in mouse brain and heart slices. J. Pharm. Pharmacol. 23: 276-279.

ROSS, S.B., RENYI, A.L. AND BRUNFELTER, B. (1968). Cocaine-sensitive uptake of sympathomimetic amines in nerve tissues. J. Pharm. Pharmacol. 20: 283-288.

SALT, P.J. (1972). Inhibition of noradrenaline $Uptake_2$ in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. Eur. J. Pharmacol. 20: 329-340.

SHORE, P.A., BUSFIELD, D. AND ALPERS, H.S. (1964). Binding and release of metaraminol: mechanism of norepinephrine depletion by α-methyl-m-tyrosine and related agents. J. Pharmacol.exp.Ther. 146: 194-199.

SKOU, J.C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membranes. Physiol. Rev. 45: 596-617.

STRÖMBLAD, B.C.R. AND NICKERSON, M. (1961). Accumulation of epinephrine and norepinephrine by some rat tissues. J. Pharmacol. exp. Ther. 134: 154-159.

SUGRUE, M.F. AND SHORE, P.A. (1969). The mode of potassium action on the adrenergic neuron amine transport system. Life Sci. 8: 1337-1341.

TAINTER, M.L. (1929). Comparative effects of ephedrine and epinephrine on blood pressure, pulse and respiration with reference to their alteration by cocaine. J. Pharmacol. exp. Ther. 36: 569-594.

TAINTER, M.L. AND CHANG, D.K. (1927). The antagonism of the pressor action of tyramine by cocaine. J. Pharmacol. exp. Ther. 30: 193-207.

THOENEN, A., HÜRLIMANN, A. AND HAEFELY, W. (1968). Mechanism of amphetamine accumulation in the isolated perfused heart of the rat. J. Pharm. Pharmacol. 20: 1-11.

TISSARI, A.H., SCHÖNHÖFER, P.S., BOGDANSKI, D.F. AND BRODIE, B.B. (1969). Mechanism of biogenic amine transport. II. Relationship between sodium and the mechanism of oubain blockade of the accumulation of serotonin and norepinephrine by synaptosomes. Mol. Pharmacol. 5: 593-604.

TITUS, E.O. AND SPIEGEL, H.E. (1962). Effect of desmethylimipramine (DMI) on uptake of norepinephrine-7-^{3}H(NE) in heart. Fed. Proc. 21: 179.

TRANZER, J.P. AND THOENEN, H. (1967). Ultramorphologische veränderungen der sympathischen Nervenendigunden der Katze nach Vorbehandlung mit 5- und 6-Hydroxy-Dopamin. Arch. Pharmak. exp. Path. 257: 343-344.

TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. Pharmacol. Rev. 15: 225-276.

TRENDELENBURG, U. (1972). Classification of sympathomimetic amines. Handb. der Exp. Pharmak. 33: 336-362.

TRENDELENBURG, U., GOMEZ ALONZO DE LA SIERRA, B. AND MUSKUS, A. (1963). Modification by reserpine of the response of the atrial pacemaker to sympathomimetic amines. J. Pharmacol. exp. Ther. 141: 301-309.

TRENDELENBURG, U., MUSKUS, A., FLEMMING, W.W. AND GOMEZ ALONZO DE LA SIERRA, B. (1962a). Modification by reserpine of the action of sympathomimetic amines in spinal cats; a classification of sympathomimetic amines. J. Pharmacol. exp. Ther. 138: 170-180.

TRENDELENBURG, U., MUSKUS, A., FLEMMING, W.W. AND GOMEZ ALONZO DE LA SIERRA, B. (1962b). Effect of cocaine denervation and decentralization on the response of the nictitating membrane to various sympathomimetic amines. J. Pharmacol. exp. Ther. 138: 181-193.

WAKADE, A.R. AND FURCHGOTT, R.F. (1968). Metabolic requirements for the uptake and storage of norepinephrine by the isolated left atrium of the guinea pig. J. Pharmacol. exp. Ther. 163: 123-135.

WHITBY, L.G., AXELROD, J. AND WEIL-MALHERBE, H. (1961). The fate of ^{3}H-norepinephrine in animals. J. Pharmacol. exp. Ther. 132: 193-201.

WHITBY, L.G., HERTTING, G. AND AXELROD, J. (1960). Effect of cocaine on the disposition of noradrenaline labelled with tritium. Nature (Lond.). 187: 604-605.

WHITE, T.D. AND KEEN, P. (1970). The role of internal and external Na^+ and K^+ on the uptake of ^3H-noradrenaline by synaptosomes prepared from rat brain. Biochim. Biophys. Acta. 196: 285-295.

WILKINSON, G.R. AND BECKETT, A.H. (1968). Adsorption, metabolism and excretion of the ephedrines in man. I. The influence of urinary pH and urine volume output. J. Pharmacol. exp. Ther. 162: 139-147.

WOLFF, J. (1960). Thyroidal iodine transport. I. Cardiac glycoside and the role of potassium. Biochim. Biophys. Acta. 38: 316-324.